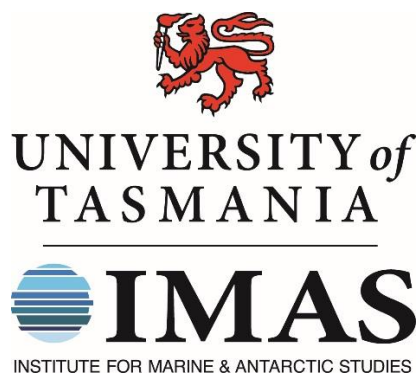


# **Cracking the code: Defining roe quality of the long-spined sea urchin (*Centrostephanus rodgersii*) in Tasmania**

**By**

**Travis Baulch**

**Submitted in fulfilment of the requirements for the  
Degree of Master of Science**



**June 2018**

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## Abstract

In Tasmania, arrival of the long-spined sea urchin (*Centrostephanus rodgersii*) has presented economic opportunity along with ecological change where they occur. Over the last half century, *C. rodgersii* has undergone climate driven range-extension and is now distributed along the entire east coast of Tasmania. The highest densities of *C. rodgersii* occur in northern Tasmania around the St Helens region and become less abundant along latitudinal gradients. This pattern of distribution has resulted in a developing fishery for *C. rodgersii* being mostly focused between ‘The Gardens’ and ‘St Helens Island’ in the north east of Tasmania where catch rates are greatest. However, a lack of definitive information on the specific drivers of roe quality has been a significant hurdle to progress of the fishery in Tasmania and mainland Australia as a whole. This species is frequently implicated with high proportions of poor quality roe in commercial catches which has deterred interest from prospective entrants to the fishery. Identifying key parameters around high quality roe will help commercial fishers and processors target individual sea urchins with a greater probability of harvesting high quality roe hereby increasing economic return with the ultimate aim of maximising the potential of this new resource.

This thesis explores the biological and environmental drivers of roe quality to determine factors that indicate high quality roe. Samples of *Centrostephanus rodgersii* were collected monthly from St Helens kelp and barrens habitats over an 18 month period spanning May 2014 to October 2015 and assessed for roe quality. Seasonal changes in reproductive biology were determined and assessed for effect on roe quality which comprised; colour, texture, granularity and quality index (possible score of 1-5 where 1 is the lowest score attainable and 5 is the highest, quality index was the sum of all individual criteria and ranged from 3-15). Examination of gonad histology showed a distinct annual pattern in reproduction and the highest levels of roe quality were recorded in the months prior to peak gametogenesis and spawning (i.e. during March, April, May and June of 2014 and 2015). Logistic regression showed high roe quality to be significantly affected by the proportion of nutritive phagocytes (NP’s) within the gonad lumen ( $G^2_I = 47.864$ ,  $P < 0.001$ ) being 4.3 times more likely (95%  $CI = 1.647, 8.412$ ) to encounter ‘A’ grade roe when NP’s were in high proportions. Furthermore, gender was also found to significantly affect likelihood of harvesting ‘A’ grade roe, but it was the interaction between proportions of NP’s and

gender that increased the odds ratio to a highly probable 9.67 to 1 (95% CI = 1.976, 58.316) when individuals were male and contained a high proportion of NP's. Histological samples collected over an 18 month period in St Helens from 2003-05 (before commercial harvesting began in Tasmania) was made available for comparative analysis and showed remarkable gametogenic synchrony with samples collected during 2014-15. Both study periods demonstrated clear winter/spring spawning (August/September) with a small number of individuals persisting through to October/November. Spawning assays (response to KCl injection) were also conducted on separate sea urchins and indicated that the magnitude of spawning response was proportional to the density of NP's. This methodological procedure may be useful as a proxy measure of roe quality for industry (i.e. signal the end of the annual high quality period) considering high quality roe is significantly affected by the presence of NP's. To this end, the decadal consistency in the seasonal gametogenic cycle and the relationship between 'A' grade roe and NP's clearly demonstrates a temporally consistent harvest window which is critical knowledge for further development of this fishery.

In addition, a suite of independent variables (exogenous and endogenous) were assessed for their effect on roe quality criteria using an ordinal logistic regression approach. Models were developed based on variables that could be; used in planning harvest operations, directly observed during harvest operations, manipulated post harvest or optimised through repeated harvesting. It was found that the independent variables; habitat, seasonality, age and test diameter (shell width) had the most influence on roe quality. Odds ratios (OR) for high quality roe were highest between April and June (OR 7.85 95% CI 4.97, 10.36) for seasonality and for sea urchins aged between 7-20yrs (OR 14.34 95% CI 9.67, 28.46). Odds ratios for sea urchins harvested from kelp habitats were also significantly increased (OR 4.78 95% CI 1.99, 4.87). The magnitude of odds ratios (particularly age) indicate that large improvements in roe quality are possible by tailoring harvest operations to accommodate these specific parameters.

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## **Chapter 1: Overview, general introduction and thesis structure**

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## **1.1 Overview**

Understanding the environmental and biological drivers of roe quality in commercially harvested sea urchins is important to maximise the proportion of high quality roe harvested. However, the mechanisms that drive roe quality for many sea urchin species are poorly understood. Overall, these mechanisms have been linked to various exogenous and endogenous factors associated with basic biological function and revolve around reproductive development.

In this chapter, the taxonomy, anatomy and distribution of *Centrostephanus rodgersii* is outlined along with the ecology, feeding behaviour, reproductive patterns and the various factors thought to influence roe quality across sea urchin species. In addition, a summary of the status of global/Australian sea urchin fisheries and markets is presented with consideration of *C. rodgersii* fishery development potential. Finally, the information presented in this chapter forms the foundation for thesis objectives and aims which are also detailed.

## **1.2 Australian sea urchins and taxonomy of *Centrostephanus rodgersii***

There are over 220 sea urchin species reported in Australia (Miskelly, 2002). The major commercial Australian sea urchin species inhabit the temperate regions where three species are most abundant (i.e. *Heliocidaris erythrogramma*, *Heliocidaris tuberculata*, and *Centrostephanus rodgersii*). The long-spined sea urchin (*C. rodgersii*) (Agassiz 1863) is the largest of the commercial sea urchin species in Australia and ecologically dominant where they occur. *C. rodgersii* (Phylum: Echinodermata) is one of few temperate sea urchin species belonging to the family Diadematidae.

## **1.3 *Centrostephanus rodgersii* anatomy**

Unlike other echinoderms, all species of sea urchins have a rudimentary anatomical structure with pentameral symmetry and their basic body configuration is encased by a calcareous exoskeleton (also known as ‘test’) that is completely surrounded by spines (Ziegler et al. 2008). These spines are the primary physical defense mechanism and are attached to the test by ball and socket joints that allow the

spines to move in all directions (Figure 1). In between spines on the ventral surface are tube feet that are used for substrate adhesion, movement and foraging. Other major external features include the dorsal anus, and ventral mouth which is surrounded by the peristomial membrane.



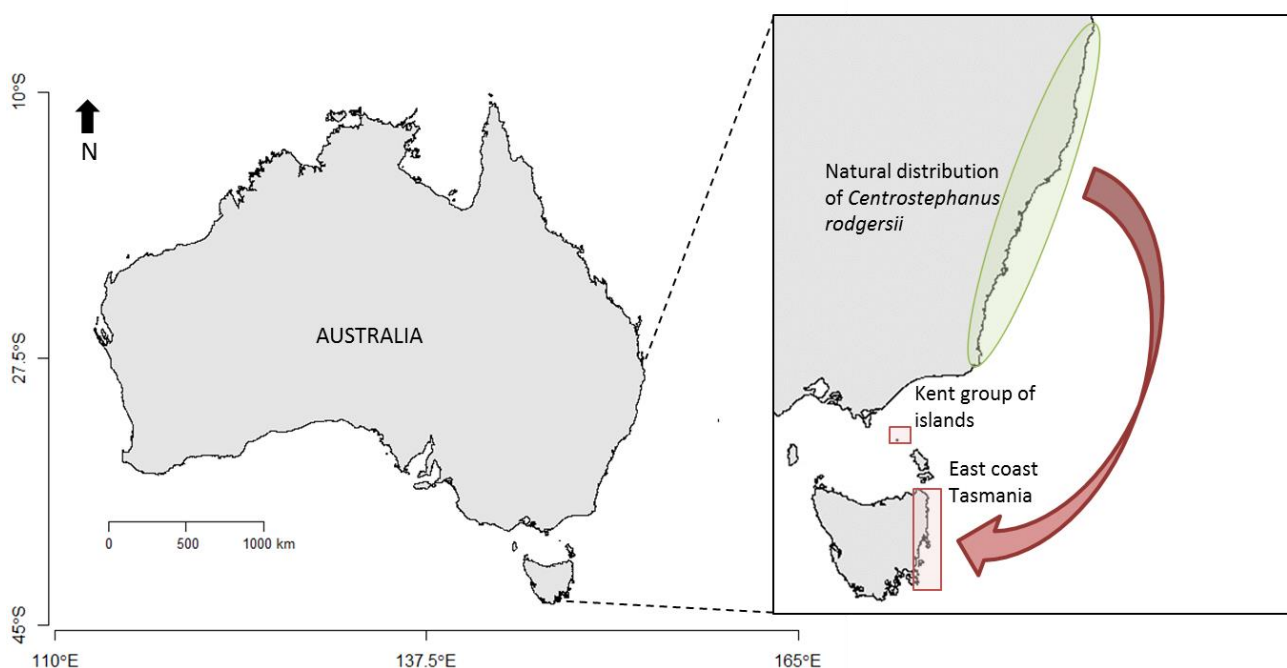
**Figure 1:** *Centrostephanus rodgersii* encased by long spines which are used as the primary defence mechanism.

Internally, the jaw structure (Aristotle's lantern) is centrally located and comprises of five teeth that extend to slightly protrude from the mouth. Food items are grazed by the teeth and passed through the Aristotle's lantern to the gut for processing. The gut and digestive tract are directly connected to the anus which expels waste. Surrounding the gut and digestive tract are five gonads that periodically dominate the internal cavity. Sea urchins of all species do not possess a brain, however utilise a very simple nervous system.

#### **1.4 Distribution of *Centrostephanus rodgersii***

*Centrostephanus rodgersii* is found on subtidal rocky substrates throughout south-eastern mainland Australia from the warm subtropical areas of northern NSW, to eastern Victoria in the south (Figure 2). They also occur off coastal Australia in Norfolk Island, Lord Howe Island, eastern Bass Strait Islands

and further afield in the northern regions of New Zealand and the Kermadec Islands (Andrew et al. 1998). However, recently *C. rodgersii* has undergone climate driven range extension and is now distributed further south to the eastern coastline of Tasmania, Australia. The southern east coast of Australia has been identified as a climate change hotspot and has recently undergone displacement of typical marine isotherm distribution as a result of a strengthening east Australian current (EAC) (Ridgway and Godfrey 1996, Thresher et al. 2004, Cai et al. 2005, Ridgway 2007). This regime shift in ocean temperature has resulted in a southward latitudinal range extension for a number of marine species through changes in dispersal patterns (e.g. Poloczanska et al. 2007, Pitt et al. 2010, Last et al. 2011, Wernberg et al. 2011) and it is likely that this has facilitated range-extension of *C. rodgersii* into Tasmania (Johnson et al. 2005; Ling and Johnson 2009; Johnson et al. 2011)



**Figure 2:** The natural distribution (green shaded area) and range-extension areas (red shaded area) of *Centrostephanus rodgersii* in south eastern Australia.

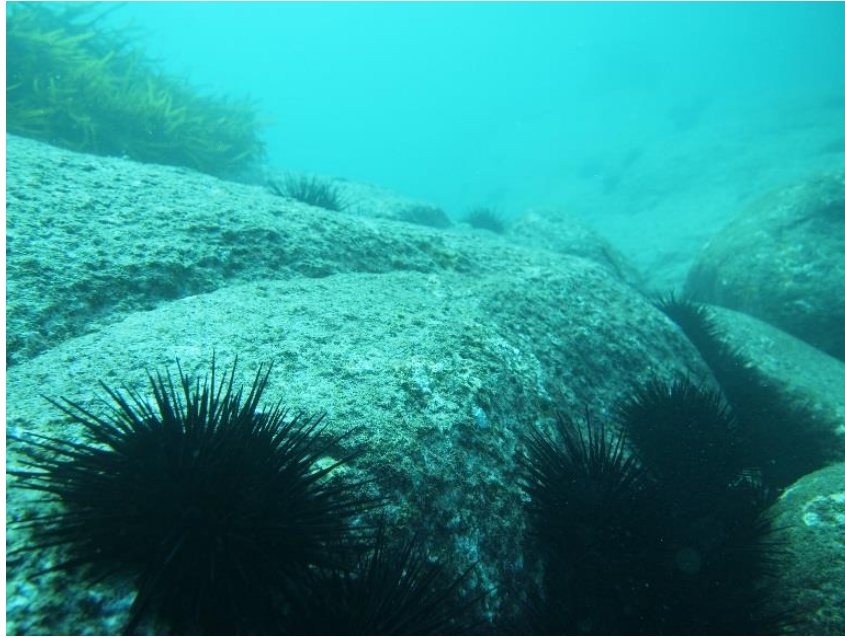
Initial observations of *Centrostephanus rodgersii* in Tasmania were documented in the Kent group of islands (Bass Strait) in the 1960's (Dartnall 1980). Since first detected in Tasmania, *C. rodgersii* has increased its southward distribution and recent studies on population dynamics and age structure

suggests significant and ongoing recruitment events have occurred in parallel to recent climate change for eastern Tasmania (Ling et al. 2008; 2009b, Johnson et al. 2011, Ling et al. 2014). Concentrations of *C. rodgersii* are highest in the north east areas of Tasmania (around the St Helens region) and become less abundant with poleward distribution (Ling and Johnson 2009).

## **1.5 Ecology and Habitat**

Throughout its distribution on the east coast of Australia, *Centrostephanus rodgersii* are seldom found in areas of high wave exposure and prefer either deeper areas or protected habitats. In its home range of NSW, *C. rodgersii* are mostly found in depths ranging from 2-20m (Andrew and Underwood 1989; Andrew and O'Neil 2000), however, occur slightly deeper (10-25m) in the range extension region of Tasmania. Below 20m, it is rare to find aggregations and individuals mostly occur among sponges and other sessile invertebrates. *C. rodgersii* are cryptic and smaller individuals seek shelter in crevices during daylight hours (Flukes et al. 2012). At night, they emerge to search for food before returning to shelter at dawn. Their foraging patterns have been shown to exhibit strong site fidelity moving a distance up to 10m and sometimes returning to the same crevice (Flukes et al. 2012). During foraging, *C. rodgersii* indiscriminately grazes a large range of macroalgal species and sessile invertebrates (Andrew and Underwood 1993; Hill et al. 2003). This feeding pattern and a voracious capacity to consume, has led to collapse of macroalgal habitats where *C. rodgersii* occur in high densities (Figure 3).





**Figure 3:** Aggregating *Centrostephanus rodgersii* in sufficient densities to create barrens that characterise certain areas in the St Helens region, Tasmania.

*Centrostephanus rodgersii* is the dominant herbivore on shallow temperate rocky reef systems across eastern Australia and is frequently implicated with major shifts in benthic ecological structure (Fletcher 1987; Andrew and Underwood 1989, Underwood et al. 1991; Andrew 1993; Ling 2008, Ling et al. 2014). Intensive overgrazing of productive kelp beds by *C. rodgersii* triggers a collapse of biogenic habitat leading to sea urchin ‘barrens’ devoid of foliose macro algal habitat. *C. rodgersii* barrens are characterised by lessened habitat complexity, productivity and biodiversity when compared to adjacent macroalgal beds that support complex ecological systems.

Reefs, whereby the entire kelp bed ecosystem has collapsed are defined as ‘extensive’ or ‘widespread’ barrens, and are formed by coalescence of smaller patch barrens (Johnson et al. 2005). Extensive barrens are not found in the shallow margins of reefs in eastern Australia (i.e. < 2m) due to wave exposure and sweeping action of large macroalgae (Johnson et al. 2005; Ling and Johnson 2009). In Tasmania, extensive barrens are known to occur at several sites around the Kent group of Islands (Bass Strait) and the St Helens area (eastern mainland Tasmania), however become less predominant in the southern extremes of distribution and only ‘incipient’ (smaller patch) barrens occur (Ling et al. 2014).

## 1.6 Reproduction and life cycle

*Centrostephanus rodgersii* has an annual reproductive cycle with major spawning occurring in late winter or early spring around August-September (Ling et al. 2008; Pecorino et al. 2012). However, spawning has also been reported to persist through to October and November (King et al. 1994; Byrne et al. 1998). Prior to spawning, gonads of *C. rodgersii* grow in size accumulating necessary nutrients required for gametogenesis. At this point, the germinal epithelium undergoes changes in reproductive biology to facilitate the development of gametes. These structural changes can be categorised according to the two major populations of cells; (a) somatic cells called nutritive phagocytes (NP's) that are present in both sexes and; (b) gender specific germinal cells (King et al. 1994). For echinoids in general, gametogenesis seasonally alters the composition and morphology of these two major populations of cells by transforming somatic NP's to reproductive cells (oogonia and spermatozoa) in preparation for spawning (Fuji 1960a, b; Pearse et al. 1986a, b; Nictora and Serafino 1988; Munk 1992; Byrne et al. 1990, 1998; Walker and Lesser 1998). Structural changes in the germinal epithelium of sea urchin gonads have been described using the reproductive staging systems of Fuji (1960 a, b) and more recently for *C. rodgersii* specifically (King et al. 1994; Byrne et al. 1998).

These staging systems simultaneously consider both populations of cells to provide a basis for understanding the cell biology of gametogenesis. The size of sea urchin gonads does not necessarily relate to the progress of gametogenesis alone. Assessment must carefully consider which cellular population (germinal or somatic) actually predominates in size and/or numbers within its germinal epithelium in order to determine the stage of gametogenesis that characterises a particular individual (Fuji 1960a, b). Typically, reproductive stages are classified into the following categories: (a) inter-gametogenesis and NP phagocytosis, (b) pre-gametogenesis and NP renewal, (c) gametogenesis and NP utilisation, and (d) end of gametogenesis, NP exhaustion and spawning. However, King et al. 1994 and Byrne et al. 1998 propose six reproductive stages for *Centrostephanus rodgersii* based on size of oocytes, amount of nutritive tissue in the germinal epithelium and propensity to partially spawn.

The process of gametogenesis in sea urchins is initiated and influenced by various environmental factors that interrelate (Kelly 2001) and can include; nutritional availability and quality (Thomson 1982; Prince 1992), photoperiod (Bay-Schmith and Pearse 1987; Pearse et al. 1986a, b; Dumont et al. 2007) and temperature (Himmelman 1986; Spirlet et al. 2000; Wangensteen et al. 2013). The environmental cues for gametogenic differentiation in *Centrostephanus rodgersii* are much less understood, however as with other diadematid sea urchins, control of reproductive activity is thought to be linked to photoperiod (Ling et al. 2008).

The seasonal changes in reproductive biology and spawning activity of *Centrostephanus rodgersii* are well described in the native home range of NSW (*see* King et al. 1994; Byrne et al. 1998). However, there is no published information describing the gametogenic changes of *C. rodgersii* in Tasmania. There have been numerous papers that describe the reproductive periodicity as a function of gonad index (GI - the ratio of gonad mass to total body mass) (*see* Ling et al. 2008; Johnson et al. 2011), but unfortunately a GI value has little further utility from a fisheries perspective because of its limited dimensions.

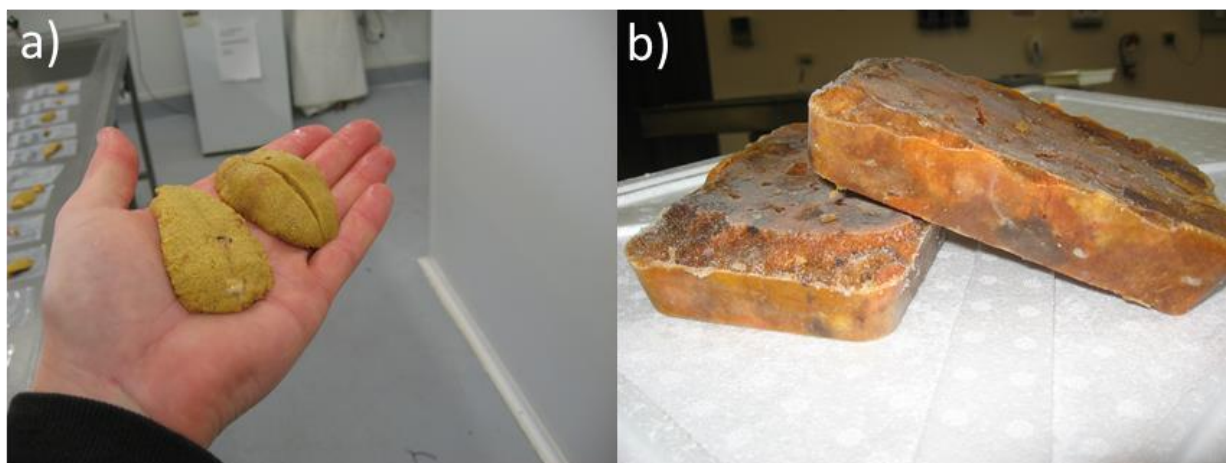
In Tasmania, at the edge of its poleward extended range, the timing of gametogenic differentiation is potentially different to that of the native range in NSW. Byrne et al. (1998) describe synchronous gonad development (i.e. GI) across seven degrees of latitude in NSW with spawning occurring between late June/July to August. However, histological examination indicated spatial differences in the timing of complete spawn-out. In Northern NSW, spawning of *C. rodgersii* may only last one month (July), whereas in the southern latitudes spawning may occur over several months concluding in November. This trend may extend to Tasmania and requires further investigation.

*C. rodgersii* to spawn synchronously (King et al. 1994) and the released gametes are fertilised in the water column to be dispersed by oceanic currents. Fertilised eggs then rapidly undergo metamorphosis into a two-armed planktotrophic larval stage that exists in the water column for ~100 days before settlement (reviewed in Ling et al. 2014). It is likely that this key feature of the *C. rodgersii* life history has facilitated southward incursion into Tasmania. Currently, there is no evidence that Tasmanian *C.*

*rodgersii* meta-populations are undergoing self-recruitment success. However, it has been found that viable gametes are produced by these populations, but development of larvae is poor at water temperatures below 12°C (Ling et al. 2008). This supports the theory that future recruitment may occur in Tasmania not only from mainland populations but also Tasmanian populations if water temperatures continue to increase.

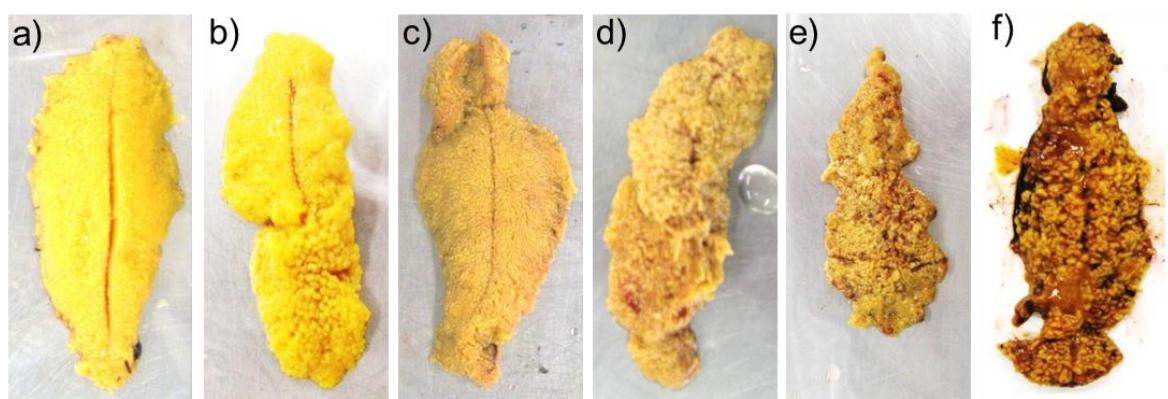
### 1.7 Roe quality criteria

Roe quality is defined by a set of market criteria usually directed by consumer demand and can include; colour, texture, granularity and taste. Roe quality attributes are qualitatively assessed immediately after processing and categorised into ‘A’ grade (highest quality possible and sold as fresh/chilled roe) or ‘B’ grade (lesser quality roe destined for market as frozen product or sauce) (Figure 4). The quality criteria of markets (particularly Japan), requires all attributes of roe quality to be optimal. Therefore, if colour and shape of roe fulfil the specific quality requirements yet granularity is coarse (undesired), the roe is relegated to lesser grades and markets.



**Figure 4:** Examples of a) ‘A’ grade roe and b) frozen roe product for lower grade markets.

According to commercial harvesters and processors in Tasmania (Mr Mead<sup>1</sup> and Mr Allen<sup>2</sup> 2014, pers. comm), there is a high degree of variation in all roe quality attributes of *Centrostephanus rodgersii*. Colour of *C. rodgersii* roe can range from highly desirable bright yellow to dark brown, the latter of which is not suitable for premium markets (Figure 5a and 5f respectively). Texture is usually assessed on the degree to which roe maintains shape/definition, and can range from fluid and disordered (Figure 5 d, e, f) to highly defined and robust (Figure 5a, b, c). Granularity ranges from a smooth gonad with small tubules to a rough gonad with large tubules (Figure 5a and 5f respectively).



**Figure 5:** Variation of *Centrostephanus rodgersii* roe quality where; a) is an example of the highest possible quality roe (bright yellow colour, fine granulation and a smooth texture); f) is an example of lowest quality roe (dark brown colour, rough granulation and coarse texture).

### 1.8 Factors affecting roe quality

Maximising roe quality in wild caught sea urchin species is a priority for commercial fishers and processors yet there is little information described in the literature. In some sea urchin species, the best roe yields are harvested when NP's have attained their greatest degree of mass increase before gametogenic differentiation is initiated (Spirlet et al. 2000; Walker et al. 2015). At this point, the NP's have accumulated the biochemical components that will be used during gametogenesis and partitioned to the developing gametes. Glycogen is the main carbohydrate stored as energy by NP's because its breakdown into glucose does not require the input of additional energy to release (Taylor et al. 2017).

<sup>1</sup> Cameron Mead, long-spined sea urchin harvest diver, St Helens, Tasmania

<sup>2</sup> David Allen, sea urchin processor (Seafoods Tasmania), St Helens, Tasmania

During early gonad growth, glycogen can range from 13% to 25% of gonad dry weight (DW) for *Strongylocentrotus intermedius* (Zalutskaya et al. 1986; Zalutskaya 1988) suggesting that glycogen could account for a substantial portion of the gonad carbohydrates. Once gametogenic differentiation is initiated, glycogen content of the gonads decline (Shpigel et al. 2004) and studies have shown that the carbohydrate content of echinoid eggs is low (Jaeckle 1995; McEdward and Miner 2007; McEdward and Morgan 2000). This indicates that the carbohydrate content of NP's is not allocated to developing gametes but rather used to provide the energy necessary to sustain gametogenesis (Zalutskaya et al. 1986). From a roe quality perspective, targeting periods when NP's (and corresponding glycogen) are most abundant means potentially harvesting sea urchin roe when it is most sweet to taste. Glycogen itself does not have a specific taste, however when consumed, the enzyme *salivary amylase* breaks down the glycogen to glucose which has a similar structure to sugar. The glucose molecules are recognised by taste receptors as sugar which implies sweetness and targeting periods when NP's (and glycogen) are most dense may result in a higher proportion of the catch with more favourable taste qualities.

Furthermore, targeting periods when NP's are most dense also means targeting periods when reproductive cells are relatively absent. Gonads that are dominated by reproductive cells have been linked to poor roe quality and relatively high levels of lipids which may contribute to a bitter taste as postulated by Phillips et al. (2010a). Also, as reproductive cells develop they grow in size and are relatively large when compared to NP's (Pecorino et al. 2009). It is postulated that large gametes may contribute to poor texture and granularity as they begin to affect the surface layers of the germinal epithelium.

Overall, the development of NP's (and consequent gametogenesis) is dependent on a number of environmental variables. However, temperature and photoperiod (seasonality) are inherently linked and shown to influence the rate of NP development in aquaculture (i.e. Walker and Lesser 1998; Garrido and Barber 2001) and in wild harvest applications (Phillips et al. 2010a). In this context, seasonality directly influences the ratio of gametogenic cells and NP's, however commercial fishing is usually tailored around periods when roe is largest (maximum return, by weight, for effort). This means

commercial fishing may be focussing effort on periods when NP's and roe quality has declined potentially affecting price at market. Although seasonality has been shown to trigger the initiation of gametogenic patterns, it is the nutritional quality and availability that determines the extent of reproductive output (Meidel & Scheibling, 1998).

In sea urchin species, the gonad is the only organ capable of storing nutrients. Their basic anatomy does not allow nutritional supply to be directly stored and drawn upon without impacting on other processes. In times when nutritive supply is low through changes in environmental parameters, sea urchins, as with other marine invertebrates, will draw energy from their gonad for nutrition and therefore basic biological function (Taylor et al. 2017). This can have significant impacts on gametogenesis which relies heavily on intra-gonadal nutrients. Thus, an understanding of the nutritional requirements for growth and development of NP's can potentially facilitate efforts to target high quality roe.

Numerous field and laboratory studies have linked food availability to roe quality (Guillou and Lumingas 1999, Guillou et al. 2000) although most of this information comes from research on sea urchins held in aquaria (Fuji 1960a, b; Himmelman 1978; McBride et al. 2004). In this context, different levels of nutrition can be attributed to contrasting sea urchin habitat types (barrens and kelp). What distinguishes and characterises these two habitat types is the level and complexity of algal cover, which is reflective of food availability and nutritional diversity for sea urchins.

In 'barrens' habitat, food is limited in availability and quality. Conversely, sea urchins in 'kelp' habitats have access to large quantities of diverse macroalgae and roe quality is expected to be high. Further to this, specific species of macroalgae contain different nutritional values and biochemical composition. If the specific dietary requirements for optimal roe quality were linked to the specific biochemical composition of particular seaweeds, it may be possible to target particular areas where optimal species of seaweeds are most abundant. Unfortunately, there has been little exploration of these hypotheses in the literature.

However, there have been attempts to enhance roe quality by transferring sea urchins from barrens habitats to either kelp habitats (Blount et al. 2002) or *in situ* experimental cages and directly feeding

kelp (Anderson and Velimirov 1982; Vadas et al. 2000). The results from these studies were promising and indicated potential gains with increases in gonad size and quality compared to sea urchins in barrens habitat. More specifically to *Centrostephanus rodgersii*, a study by Blount et al. (2017) investigated the potential of roe quality enhancement by reducing densities in barrens or transplanting individuals to kelp habitats in NSW. The study noted significant improvements in yield and colour of roe over short periods of time (i.e. 3 months) and greater improvements over two years, although the magnitude of change was also dependent on density and season.

Overall, nutrition has been shown to directly influence the quality of roe, particularly so when coupled to the smaller sea urchin size classes. A study by Blount and Worthington (2002) found that *Centrostephanus rodgersii* (in the home range of NSW) with smaller test diameter (TD) and from kelp habitats were more likely to contain roe of a preferred colour. There are few other examples of sea urchin size relating to roe quality in other species (e.g. Pearce et al. 2004; Woods et al. 2008). However, size is generally representative of age within habitats, and age may play a significant role with roe colour. A study by Agatsuma (2005) found age to cause brown coloration in roe of *Strongylocentrotus nudus* where older individuals contained darker, less preferred roe. These studies suggest that targeting small and potentially young sea urchins from kelp dominated habitats may result in better overall quality of roe.

At the conclusion of harvesting attention is usually turned towards care and optimised processes for sea urchins. Post-harvest handling and processing have been shown to influence roe quality and shelf-life of the New Zealand urchin (*Evechinus chloroticus*) (Verachia et al. 2012, 2013). Published information is not available for other sea urchin species. In Australia, sea urchins can be harvested significant distances from processing facilities and as such, may be held in ambient air temperatures for 24hrs to 48hrs. While the impact of this standard commercial practise has not been reported for *Centrostephanus rodgersii*, it is likely to be deleterious for roe quality.

Overall, roe quality is undoubtedly the most important factor in supplying lucrative Asian markets and therefore defining the parameters responsible must be a priority for commercial harvesters and



processors. On an individual level, *Centrostephanus rodgersii* and sea urchins in general, do not display any external indication of roe quality for outlined criteria and therefore careful consideration of available information must be given to maximise quality.

## **1.9 Major sea urchin markets**

Japan is the world's largest importer and consumer of sea urchin roe. However, smaller markets exist in the United States of America (USA), Europe, and China. The price of imported sea urchins (fresh or chilled) in Japan has been over 2 to 3 times higher than that in the USA over the last 30 years (FAO, fishstat) and therefore many sea urchin fisheries around the world are focused on supplying this market. Japan imports approximately 90% of global sea urchin products (FAO, fishstat) and arrives either whole, fresh or frozen. Prices can vary from US\$10/kg for frozen product to US\$250/kg for fresh premium roe (Andrew et al. 2002). A majority of fresh roe are sold by auction at the Tokyo Central Wholesale Market where the highest quality roe are neatly packed in traditional wooden trays with individual segments neatly presented. The highest quality roe (termed 'Uni' by the Japanese market) is consumed raw as sashimi or served with rice as sushi (Andrew et al. 2002).

Traditionally, the seasonal availability of sea urchin products in Japan has been closely linked to the reproductive cycle of *Strongylocentrotus intermedius* and *S. nudus*. These species comprise 80% of total supply to the domestic Japanese market and are mostly harvested between March and August. However, more recently the Japanese population has come to expect year round supply of sea urchin products and the highest prices are usually paid in January and September, reflecting the low availability of domestic roe during these months (Sonu, 1995). This coupled with declining local stocks has driven the Japanese market to source overseas species of sea urchin. Apart from the local Japanese product, roe shipped from Canada and the USA are considered to be the best in Japan's import market, followed by roe from Mexico, Russia and South Korea (Sonu, 2003). The future market direction for imported sea urchins to Japan depends largely on the Japanese sea urchin harvest. Because the domestic harvest is not likely to increase in the short term, increased export of sea urchin products from around the globe has significant potential.

### 1.10 Global sea urchin fisheries

Sea urchins have been part of subsistence fisheries on the northern Atlantic coastlines, northern Asia (principally Japan and Korea), and Chile for centuries. However, these traditional fisheries have subsequently been commercially exploited and now account for a large proportion of total global production. Catch landing records show annual output of global sea urchin fisheries has steadily increased from the early 1950's until a production peak in 1995 of approximately 120,000t (Kesting and Hall 1998; Andrew et al. 2002). Since then, catches have declined substantially and plateaued to current levels of 60,000-70,000t per annum (FAO fishstat). During the last 10 years, there have been 26 countries that have landed sea urchins with a majority of catch coming from; Japan, Chile, USA, Canada, South Korea, Russia, Mexico (Andrew et al. 2002). Japan has historically produced the largest global landings of sea urchins reaching a production peak of 26,000 t in the late 1960's (Andrew et al. 2002). However, during the 1980's, Japanese landings began a precipitous decline reaching lows of approximately 13,000t (Andrew et al. 2002). This decline triggered a dramatic change in the world market by increasing Japanese demand for alternative supplies of sea urchin products. In this context, Chile became a significant producer of sea urchin roe. Currently, a majority of the annual global production is harvested from Chile which consistently produces over half of the world's annual total landings. Prior to 1975, the total landings was <3000t per year. The fishery then entered a phase of rapid expansion and catches grew by 2800 t per year until a production peak of 54,000t in 1995 (Moreno et al. 2007). However, as more accessible northern areas of the Chilean coastline became depleted, fishing effort shifted into the southern unfished areas. Sea urchins are now harvested from the full length of the Chilean coastline from the border with Peru to Cape Horn. The fishery is now considered fully exploited and in probable decline (Andrew et al. 2002).

California began developing a sea urchin industry in the early 1970's (similar to Chile), based on abundant supplies of red sea urchin (*Strongylocentrotus franciscanus*) found mainly near the Channel Islands off Los Angeles (Botsford et al. 2004). However, a majority of these landings were for domestic markets as ex-pat Japanese business' (restaurants and sushi bars) began to develop in Southern California. Throughout the 1970's annual landings grew slowly and reached 10, 000 t by the end of the

decade (Kalvass and Hendrix 1997; Richards et al. 1998). Around the same time as the development of the Californian fishery, the east coast of North America (Maine, New Brunswick and Nova Scotia) was exploring the potential of a fishery for the green sea urchin (*Strongylocentrotus droebachiensis*). Initially, small quantities were harvested for local ethnic communities. However, much like the patterns witnessed in Chile and California, Maine's industry grew at an accelerating pace reaching a level of 18,600 t in 1993 which was soon followed by drastic reductions in total catch landed (Perry et al. 2002). Overall, in an attempt to consistently supply lucrative Asian markets, large-scale commercial harvesting and spatial expansion around the globe has led to sequential depletion in many of these once productive areas (Lesser and Walker 1998; Berkes et al. 2006). The consequent decrease in global supply of sea urchin product from these fisheries has driven markets to further explore the potential of other sea urchin fisheries that were previously considered inferior to the traditionally preferred species of Japan and the American continents.

### **1.11 Australian sea urchin fisheries**

In Australia, there have been several attempts to develop sea urchin fisheries, yet the total annual catch remains low. During 2015, a modest 202 t was harvested in Australia which represents only a fraction of global production (~0.07%). Small-scale harvesting occurs in the south-east temperate regions of Australia including; New South Wales (NSW), Victoria, Tasmania and South Australia. These fisheries are focused on three main species including; *Heliocidaris erythrogramma*, *H. tuberculata* and *Centrostephanus rodgersii*. There is overlap in the distribution of these species and volumes caught are different in each region. In Tasmania, two sea urchin species are commercially harvested (*Heliocidaris erythrogramma* and *Centrostephanus rodgersii*). Historically the Tasmanian catch has consisted of only *H. erythrogramma*. However, over the last decade *C. rodgersii* has become the major species of sea urchin harvested (Mr Ryan <sup>3</sup>2014, pers. comm).

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<sup>3</sup> Greg Ryan, senior fisheries manager (commercial dive), Department of Primary Industries, Parks, Water and Environment, Tasmania

Commercial harvesting of *Centrostephanus rodgersii* began in Tasmania during the 2009/10 season (7.51 t) and annual landings grew quickly to 95.5 t in 2013/14. However, these volumes have since retracted (39 t during 2015/16 harvest season) due to the major sea urchin processing facility ceasing operations. Currently, there are no size or total allowable catch (TAC) limits for *Centrostephanus rodgersii* in Tasmania.

## **1.12 Thesis outline**

The objective of this thesis is to improve understanding of the complex interactions between the various biological and environmental parameters that drive roe quality in *Centrostephanus rodgersii*. Ultimately, the research presented here is designed to increase the probability of harvesting high quality roe to improve the economic viability of the fishery. To do this, Chapter 2 identifies the gametogenic patterns of *C. rodgersii* from histological preparations of gonads collected across the east coast of Tasmania during 2003/2005 to outline any spatial differences in the timing of gametogenesis. This information was then compared to more recent data collected ~10 years later (between 2014/2015) at St Helens to determine any temporal differences in the timing of gametogenesis. The second part of this chapter assesses the relationship between NP's and roe quality with the overall aim to understand the magnitude of effect of NP's on roe quality to outline the optimal harvest window in eastern Tasmania.

Chapter 3 investigates the various environmental and biological factors that contribute to roe quality. This chapter sets out to identify the main factors responsible for roe quality. Based on published literature, it is hypothesised that contributing independent variables such as; seasonality, age, and habitat type play a significant role with roe quality of sea urchins. *Inter alia*, these factors are used for the development models to identify significant effects within each independent variable.

These chapters combined provide fundamental information to potentially maximise roe quality harvested. The conclusions from this work will assist commercial harvesting operations and provide valuable insights to inform harvest operations and maximise roe quality to improve the economic viability of the fishery.



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**Chapter 2: Effects of seasonal reproduction on roe quality for the long-spined sea urchin (*Centrostephanus rodgersii*) in Tasmania**

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## 2.1 Abstract

The sea urchin *Centrostephanus rodgersii* has recently undergone climate driven range-extension to Tasmania. However development of this new resource has been slow. Roe quality of *C. rodgersii* is notoriously variable and optimal harvest windows are defined here for advancements of the fishery. Examination of roe histology over an 18 month period spanning May 2014 to October 2015 in St Helens north-eastern Tasmania, showed a distinct seasonal pattern and the highest levels of roe quality were recorded in the months prior to peak gametogenesis and spawning. Logistic regression analysis showed that high proportions of nutritive phagocytes (NP's) significantly affect the odds ratio of harvesting 'A' grade roe ( $G^2 = 47.864$ ,  $P < 0.001$ ) being 4.3 times more likely (95% CI = 1.647, 8.412). In addition, gender was found to significantly affect the chances of harvesting 'A' grade roe ( $G^2 = 16.349$ ,  $P < 0.001$ ). However, it was the interaction effect of gender (males had higher proportion of 'A' grade roe) and nutritive phagocytes ( $G^2 = 7.466$ ,  $P < 0.001$ ) that increased the odds ratio (OR) of harvesting 'A' grade roe to a highly probable OR 9.67 : 1 (95% CI = 1.976, 58.316). Histological samples collected well before the advent of the fishery in eastern Tasmania (i.e. during an 18 month period from 2003-05), but for which roe quality from a fisheries perspective was not assessed, showed remarkable gametogenic synchrony when compared to histological patterns from 2014-15. All study populations demonstrated clear winter/spring spawning periods with major gamete release beginning in August/September and relict gametes present through to October/November. Spawning response to potassium chloride injection (KCl) is shown here to be a useful proxy indicator of proportional roe quality and a potential 'real time' signal to the end of the high roe quality period. The decadal consistency in the timing of gametogenic development and the relationship between 'A' grade roe and nutritive phagocytes (NP's) clearly demonstrates a spatially and temporally consistent harvest window which is critical knowledge for further development this fishery.

## 2.2 Introduction

Sea urchins are harvested for their gonad (termed ‘roe’) and considered one of the most valuable seafood products in Asian markets. Prices for fresh roe vary widely depending on; origin, supply/demand, species, and most of all quality. There are various exogenous and endogenous factors that are thought to influence roe quality yet the parameters for most species remain undefined. Identifying these drivers of quality is required to consistently supply markets with high quality roe for maximum economic return.

Consumers in Asia are well known for their enthusiasm to pay high prices for high quality seafood products. However, these markets are particular about quality and have high standards when assigning ‘high quality’ to seafood products. In general, the highest prices are paid for roe that is firm, small (<5cm length), free of leaking fluids and brightly coloured (Blount and Worthington 2002; Sonu 2003; McBride et al. 2004; Phillips et al. 2010b). Species of the genera *Strongylocentrotus* are widely perceived by Asian markets to be indicative of high quality and therefore attract the highest prices. However, as these species have become harder to source through localised depletion, this has presented market opportunities for lesser exploited species.

In Tasmania, the sea urchin *Centrostephanus rodgersii* is a relatively new arrival and has undergone range-extension from northern latitudes (i.e. NSW and eastern Victoria). This species has increased in abundance and now occurs in high “commercially harvestable” densities presenting a new commercially exploitable resource. However, the parameters around roe quality are yet to be defined which is problematic for maximising potential of this new resource.

When determining strategies for the commercial harvest of sea urchins, seasonality is usually the first consideration. Roe is the primary reproductive organ of sea urchins and known to vary in size (and quality) throughout the seasonal reproductive cycle (Himmelman et al. 1978; Meidal and Scheibling 1998; Phillips et al. 2010a). Therefore, many sea urchin fisheries are focused on periods when roe is largest to maximise return for effort. However, this period is typically associated with mature gonads dominated by fully developed gametes and can lead to poorer quality roe. Gametogenesis routinely



alters the reproductive biology within gonads of echinoids transforming somatic nutritive phagocytes (NP's) to reproductive cells (oogonia and spermatozoa) in preparation for spawning (Fuji 1960a, b; Pearse et al. 1986a, b; Nictora and Serafino 1988; Munk 1992; Byrne et al. 1990, 1998; Walker and Lesser 1998). NP's have been linked to high roe quality in some sea urchin species (Spirlet et al. 2000; Walker et al. 2015).

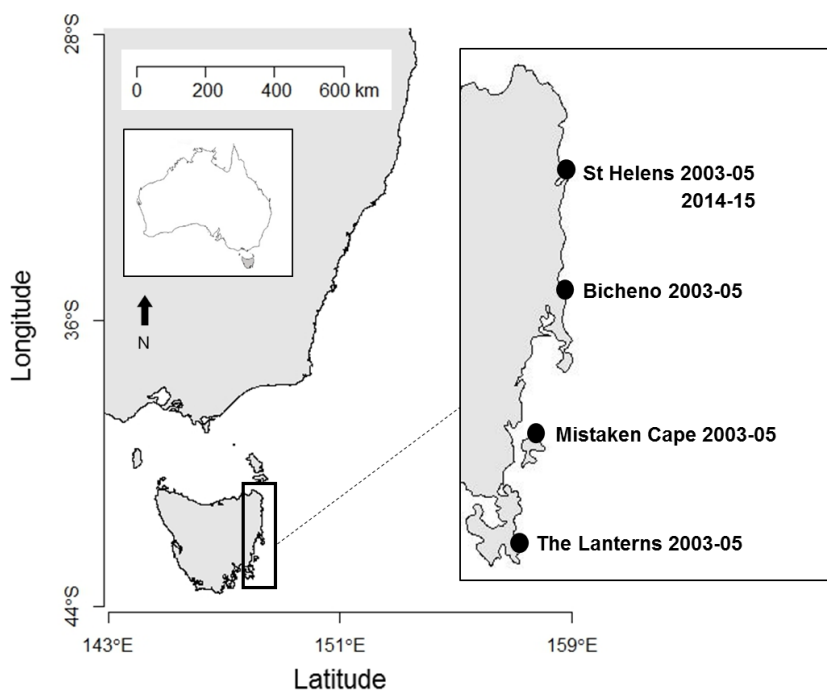
It is postulated that targeting periods when NP's dominate the gonad prior to gametogenesis may improve the probability of harvesting high quality roe of *Centrostephanus rodgersii* in Tasmania. However, gametogenic patterns of *C. rodgersii* in Tasmania at the edge of its poleward extended range are potentially different to that of the native range in New South Wales (NSW) which would affect the timing of spawning and possible optimal 'harvest windows' (HW). As such, we assess the potential of the Tasmanian fishery by identifying temporal and spatial changes in reproductive biology where high quality roe may constitute a large proportion of the harvest. Here we examine the reproductive cycle of *C. rodgersii* in two discrete periods 2003-05 (prior to the sea urchin fishery where histological samples were taken but before roe quality was measured) and 2014-15 (5-6 years into the fishery, when both histological and roe quality was measured) to determine seasonality of the reproductive cycle and roe quality; with the aim of defining the optimal HW.

## **2.3 Methods**

### **2.3.1 General**

Samples of whole *Centrostephanus rodgersii* were collected from four sites during 2003-05 (bimonthly) and one site during 2014-15 (monthly) (Figure 6). Sites were spread along ~200 km of the Tasmanian east coast within the north eastern and central eastern commercial dive fishery management zones. Distance between research sites varied from 50km-80km encompassing the majority of *C. rodgersii* distribution in Tasmania (*see* Johnson et al. 2005). Study sites included Elephant Rock, St Helens (41°14'56"S; 148°20'18"E); The Gulch, Bicheno (41°52'29"S; 148°18'27"E); Mistaken Cape, Maria Island (42°38'34"S; 148°9'17"E); and The Lanterns, Tasman Peninsula (43°8'19"S; 148°0'21"E) for

2003-05. Samples collected in 2014-15 were collected from Skeleton Point, St Helens (41°14'54"S; 148°19'46"E). This site was selected at St Helens because it was not possible to obtain samples from the Elephant Rock Research Reserve due to an ongoing research project (FRDC Project No. 2007/045) that prohibited the extraction of *C. rodgersii*. The (2014-15) Skeleton Point site is separated by ~500m from the (2003-05) Elephant Rock site and both are north-east aspect (same exposure) with the same distribution of macroalgal assemblages (distinct separation at ~12m depth dividing barren habitat devoid of seaweed and healthy kelp habitat to the shallower margins). Collections at Skeleton Point were monthly with the exception of November (2014) and January (2015) due to weather and logistics. All collections were conducted in kelp habitat using SCUBA from depths ranging 8-10m.



**Figure 6:** Four study sites along the east coast of Tasmania where *Centrostephanus rodgersii* were sampled bimonthly during 2003-05 and monthly during 2014-15.

### 2.3.2 Seasonality in Gonad Somatic Index

Sea urchins were collected in the size range of 75 – 120 mm test diameter (TD) to represent typical commercial catch size-frequency and only target mature animals, which also reduces potential size

related biases in gonad somatic index (GI). Individual sea urchins were measured (TD) using digital callipers to the nearest 0.1 mm, weighed (precision 0.1 g), drained of coelomic fluid plus any free-surface water and dissected within 24hrs. Gonads were excised and weighed and GI was calculated as:

$$GI(\%) = \frac{\text{Total gonad weight (g)}}{\text{Total body weight (g)}} \times 100$$

### **2.3.3 Roe quality**

The St Helens area was selected for roe quality assessment as this region is where a majority (~95%) of *Centrostephanus rodgersii* commercial harvesting occurs. Samples collected from St Helens, Skeleton Point (2014-15) for roe quality assessment were placed in fish bins and held at room temperature in the Institute for Marine and Antarctic Studies (IMAS) fish processing laboratory and processed the following morning (i.e. within 24 hrs). Roe quality was categorised for the criteria of: colour; texture; and granularity, with each given a rank score of 1-5, with 1 being the minimum and 5 being the maximum quality attainable for each category. Colour was assessed using commercial standard colour cards (DPIPWE catch report docket book) where 1 = dark brown, though to 5 = bright yellow. Texture was assessed and scaled to commercial criteria based on the degree to which roe maintained its shape and definition, where 1 = very fluid and disordered, and 5 = highly defined and robust. Granularity was assessed according to commercial processing criteria (granular surface definition) and based on the qualitative size of gonad lumen tubules (1-5). Here, 1 = a rough gonad with large tubules, and 5 = a smooth gonad with small tubules. Individual sea urchins were also categorised into 'A' grade and 'quality index' (sum of all quality metrics). 'A' grade individuals required a categorical score of 4 or above in all roe quality criteria as per commercial standards.

### **2.3.4 Gametogenesis and histochemistry**

One gonad from each individual sea urchin collected was fixed in Bouin's solution for 24hrs. To prepare the solution, 20g of picric acid was stirred into 1000ml of distilled water then mixed with 75ml aqueous picric acid, 25ml formaldehyde, and 0.5ml glacial acetic acid. After fixing, samples were preserved in

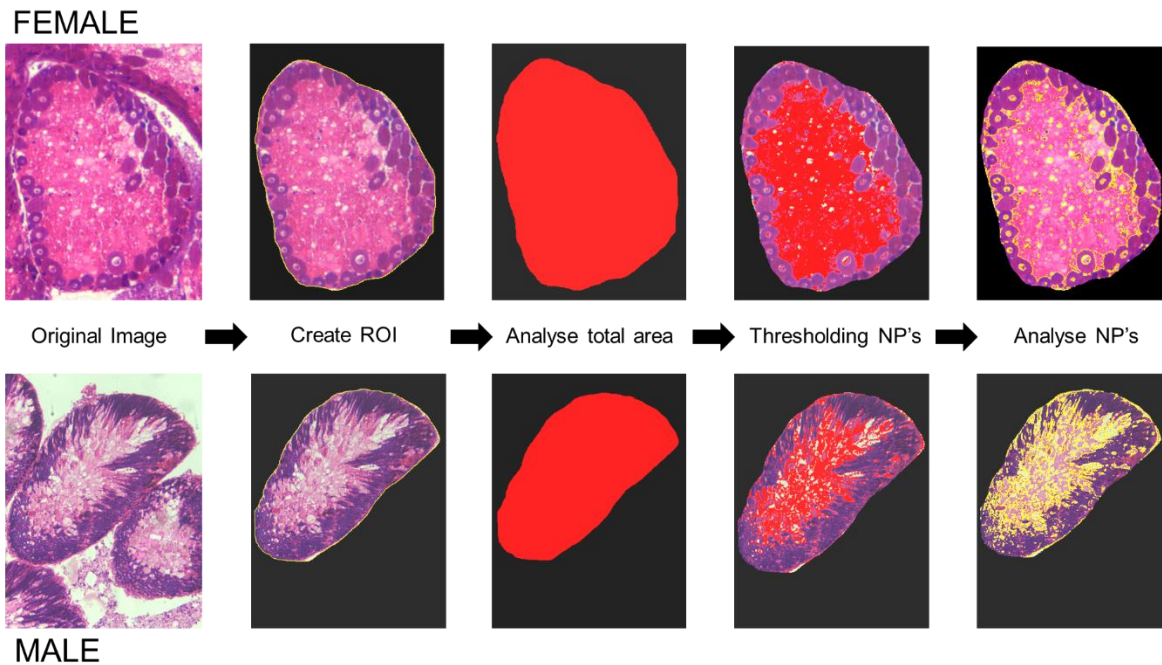
70% ethanol. Samples were embedded in paraffin, sectioned (7  $\mu\text{m}$  thick) and stained using haematoxylin (deep blue and purple that stains nucleic acids) and eosin (stains proteins to varying degrees of pink). Previous studies have shown reproductive condition to be homogeneous throughout an individual sample of *Centrostephanus rodgersii* (all five gonad lobes) and a single gonad (King et al. 1994; Byrne et al. 1998), therefore one gonad lobe per individual were used. Reproductive stages were categorised based on six maturity stages according to reproductive morphological criteria. Stages were classified as: recovering; growing; premature; mature; partly spawned; and spent (after Byrne et al. 1998; King et al. 1994).

### **2.3.5 Image analyses of histological condition**

Proportion of NP's within gonads were quantified (% area) from digital images captured using the software package 'Leica Application Suite' for microscope model Leica DM250. Images were taken from constructed histological slides and assessed using image analysis software ImageJ Version 1.51k (Schindelin et al. 2012). Methodological approach followed that defined in Arena et al. (2017) and adapted for reproductive cell biology. For assessment, a region of interest (ROI) was created using the freehand selection tool and tracing around the acini wall of an individual teste or ovary (Figure 7). The ROI was then isolated by the 'clear outside' function and colour thresholding was used to count the individual pixels within the ROI to ascertain the total area of the teste or ovary. To quantify the area of NP's within the acini, hue was adjusted using colour thresholding to highlight eosin stained NP's. The segmented area was then selected and pixels counted using the measure function. To calculate the percentage area of NP's, counted pixels of NP thresholding was divided by the total pixel count of the ROI and multiplied by 100. This process was completed in triplicate (i.e. three individual testes or ovaries per individual) to calculate mean NP percentage per individual.

This approach was validated by comparing the difference between the more traditionally used method of area measurement (manual freehand selection and multiple ROI's) to the method used herein. Five individuals per gender per reproductive stage (total n=60) were assessed using both methods (triplicate assessment per gonad). There were no significant differences in methods within reproductive stages and

genders. However, the manual freehand selection method showed lower counts on average compared to colour thresholding, presumably due to the fine scale regions of NP's that cannot be segmented manually.



**Figure 7:** Steps in image analysis of gonad histological sections of *Centrostephanus rodgersii*. From left to right; original image captured by the Leica Application Suite for microscope model Leica DM250; region of interest (i.e. singular teste or ovary) created using the freehand selection tool and clear outside function; analyse ROI for pixel count to calculate total area of teste or ovary; colour thresholding to segment NP's; select and count segmented pixels.

### 2.3.6 Spawn induction assays and natural spawning after collection

Spawn induction assays were conducted using potassium chloride (KCl) and 10 randomly selected sea urchins from the St Helens area per sample occasion. Assays were done during two discrete time periods, bi-monthly throughout the 2003-05 study period and during 2015 over a six month period. Injections were made using 4 mL of 0.5M KCl then observed for 30 minutes for evidence of spawning (i.e. gametogenic discharge from gonadopores after a period of 2-3 hours).

Separate to spawn induction assays, natural spawning after collections for the 2003-05 (bi-monthly n=15) and 2014-15 (monthly n=15) periods, were observed and recorded after a period of 2-3 hours. If

individuals showed evidence of spawning (using the same criteria as spawn induction assays), samples were categorised positive for ‘spawn on arrival’ (SOA) regardless of spawn intensity.

### 2.3.7 Design and statistical analyses

All data analyses were conducted using R 3.0.3 (R Core Team, 2014). Seasonal GI variation was examined using one-way ANOVA to compare variability within and between sites for each sampling period of 2003-05 and 2014-15. Similarity between samples collected during 2003-05 and 2014-15 were assessed between Elephant Rock and Skeleton Point (St Helens region) by Mann-Whitney-Wilcox test. To examine the seasonal variation in the proportion (% area per gonad lumen) of NP’s, one-way ANOVA was used to test significant differences between months within reproductive stages. Chi-square analyses were used to test gender ratios and the relationship of gender to individual roe quality metrics of colour, texture and quality.

The effect of gender and proportion NP’s (categorised as low ‘0-33%’, medium ‘34-66%’, high ‘67-100%’) was examined for their effect on ‘A’ grade roe. These variables were selected because of their suggested influence on roe quality (e.g. King et al. 1995, Walker and Lesser 1998; Blount and Worthington 2002, Phillips et al. 2009, Phillips et al. 2010a). Multiple logistic regression analyses were used to determine the odds ratios of the binomial response variable of ‘success’ defined as ‘A’ grade roe when considering the fixed effect of gender and random effect of proportion NP’s in the model. The model as follows;

$$\text{Logit}(y) = \mu + \alpha_i + \beta_j + \alpha\beta_{ij}$$

Where; Logit (y) = categorical response of ‘A’ grade;  $\mu$  = the intercept;  $\alpha$  = the effect of ‘gender’ ;  $\beta$  = the effect of ‘proportion nutritive phagocytes’;  $\alpha\beta_{ij}$  = the interaction effect of ‘gender : proportion nutritive phagocytes’

During 2003-05, samples were collected using a sample size of n=30. Variance in GI was estimated *post priori* over the seasonal cycle using power analysis for one-way ANOVA designs to determine a

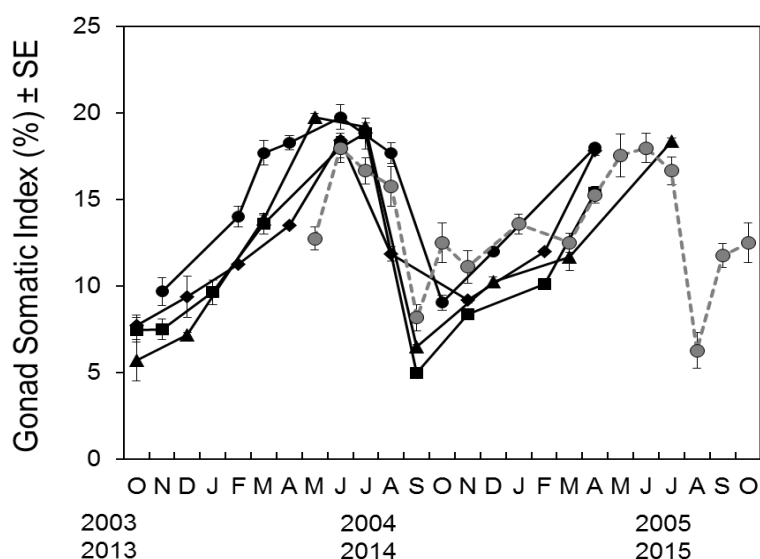
*priori* reduction in sample size for 2014-15 collections. Power analyses was conducted by constructing 4 vectors representing sites containing monthly GI group means incorporating largest monthly variance found across the GI cycle respective of site. A reduction to  $n=15$  was found to be appropriate given no loss of power and was used for 2014-15 collections. 15 replicates were randomly selected from samples collected during 2003-05 to balance the design. This practical reduction of sample size resulted in similar seasonal GI variation patterns and allowed time for roe quality assessment of 2014-15 samples. All samples were processed within 24hrs of collection. In addition, power analyses were conducted for two-way contingency table based on probabilities that we expected under  $H_A$  to gauge the effect size and estimate required sample size for logistic regression. Estimated probabilities were based on literature that demonstrates the positive/negative effect of independent variables. A sample size of  $n=200$  was found to give appropriate power. However, the experimental period was intended for 18 months and previous ANOVA power analyses determined  $n=15$  was appropriate, therefore monthly collections would exceed this estimate, and  $n=15$  was used. All power analyses were done using the 'pwr' package in the statistical program 'R'.

## **2.4 Results**

### **2.4.1 Temporal and spatial cycles of gonad growth and gametogenesis**

Mean GI demonstrated highly seasonal patterns across all sites and decadal time series. GI slowly increased during mid-summer through to late autumn (December-May) to peak at all sites between the winter months of June and July (with the exception of The Lanterns 2003-05 which peaked in May 2004), this was followed by a sharp decline during the months of August and September. One-way ANOVA showed significant temporal variation within 2003-05 sites ( $F=110.84$ ,  $P<0.001$ ) yet no significant differences in variation between sites ( $F=1.78$ ,  $P=0.183$ ), indicating that the timing of gonad growth was similar across all sites (Figure 8). Mann-Whitney-Wilcoxon test indicated significant ( $P=0.027$ ) decadal dissimilarity in GI variation per month within the St Helens region (2003-05 and 2014-15) although GI development trends showed similar patterns overall. Mean GI across sites and

study periods ranged from 5% (Bicheno 2003-05) to 19.8% (St Helens 2003-05). Pre-spawning indices ranged from 13% to 19.8%, whereas post-spawning indices ranged from 4.8% to 11.8%.

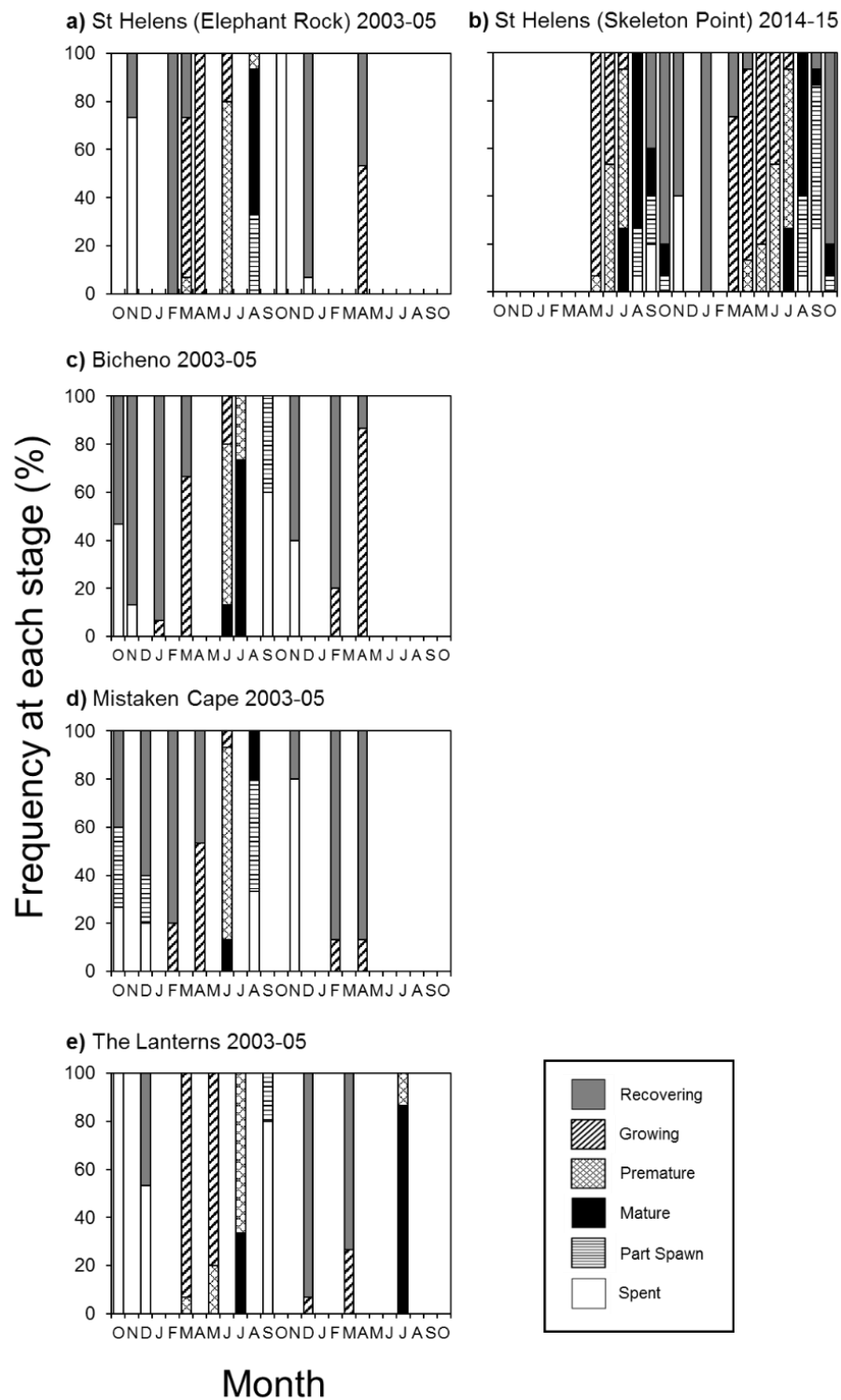


**Figure 8:** Mean gonad somatic index values ( $\pm$  SE;  $n=15$  per sampling occasion) of *Centrostephanus rodgersii* at four sites in eastern Tasmania. St Helens 2003-05,  $\bullet$ —; St Helens 2014-15,  $\circ$ —; Bicheno 2003-05,  $\blacksquare$ —; Mistaken Cape 2003-2005,  $\blacklozenge$ —; The Lanterns 2003-05,  $\blacktriangle$ —.

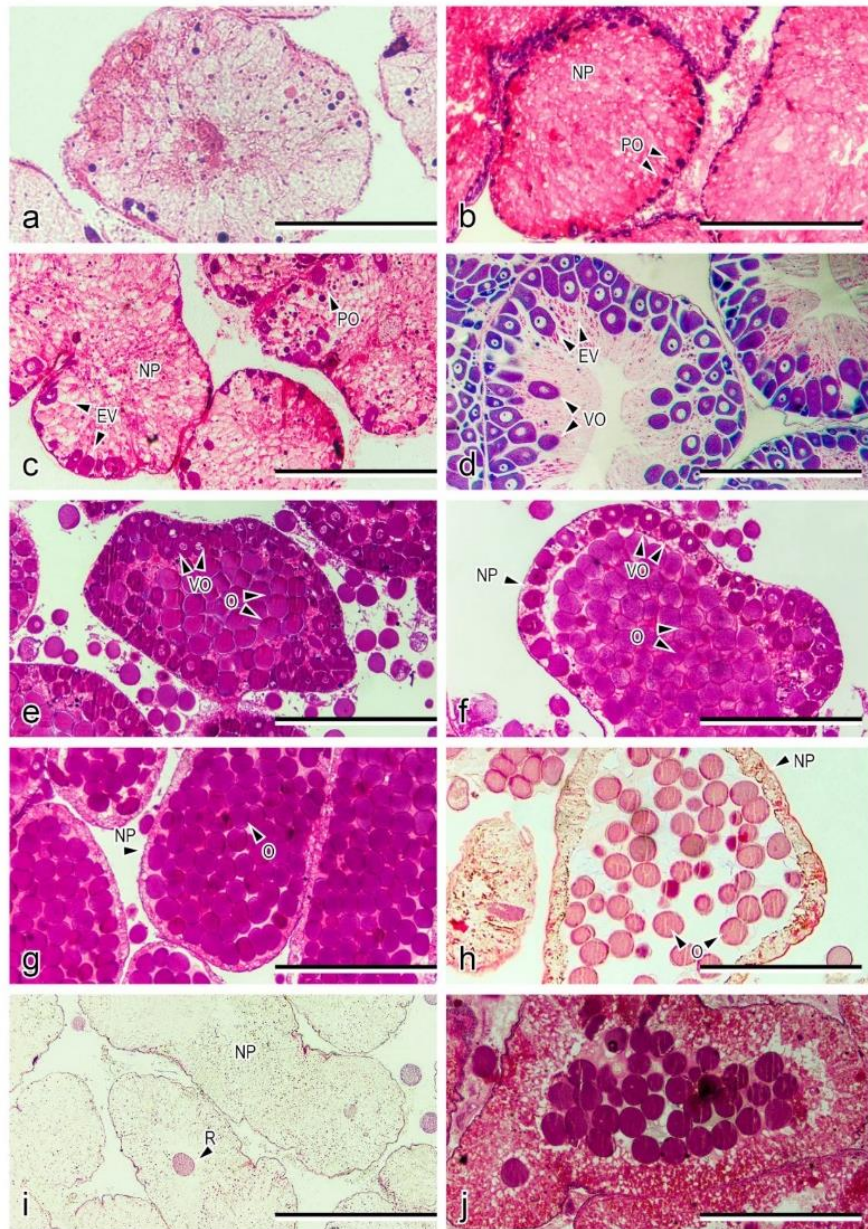
Histological assessment and categorisation of reproductive condition showed that timing of gametogenesis was highly synchronous across all 2003-05 sites and between decadal time periods (i.e. in St Helens between 2003-05 and 2014-15) (Figure 9). The major spawning period was August/September and during this time gonads were found to be mostly ‘spent’ and ‘partially spawned’ (Figure 9) which also coincided with a sharp drop in GI. After the observed major spawning period, some samples were found to contain a small amount of relict gametogenic material in the early stages of phagocytosis (Figures 10i, 11h) and this trend persisted until November. Throughout summer and spring (December – May), gonads slowly accumulated NP’s and were categorised as ‘recovering’/early ‘growing’ and were highly eosinophilic due to the strong presence of nutritive tissue (Figures 10b, 11a). During the later stages of this period, pre-vitellogenic oocytes were starting to develop in the germinal epithelium and primary spermatocyte columns were in the early stages of development (Figure 10c, 11b). Samples collected in April were mostly categorised as early and late ‘growing’ with nutritive



tissue beginning to diminish. During this time, ovaries contained vitellogenic oocytes yet there were no mature ova present (Figures 10d) and testes had well-developed spermatocyte columns (Figure 11c). June marked the onset of gametogenesis and coincided with peak GI. Histological examination indicated most sea urchins were in early and late ‘premature’ reproductive stages. During the ‘premature’ stage, there was clear definition between the central mass of gametes and the germinal layer (Figures 10e, f, 11d, e). Males showed well developed spermatocyte columns with spermatozoa forming around the tips and taking a central position within the lumen and females accumulated mature ova in the centre of the lumen. By July, gametogenic differentiation was largely complete and individuals were mostly classified as ‘mature’ with an abundance of large ova and the testes displayed large amounts of spermatozoa dominating the lumen in preparation for spawning (Figures 10g, 11f).

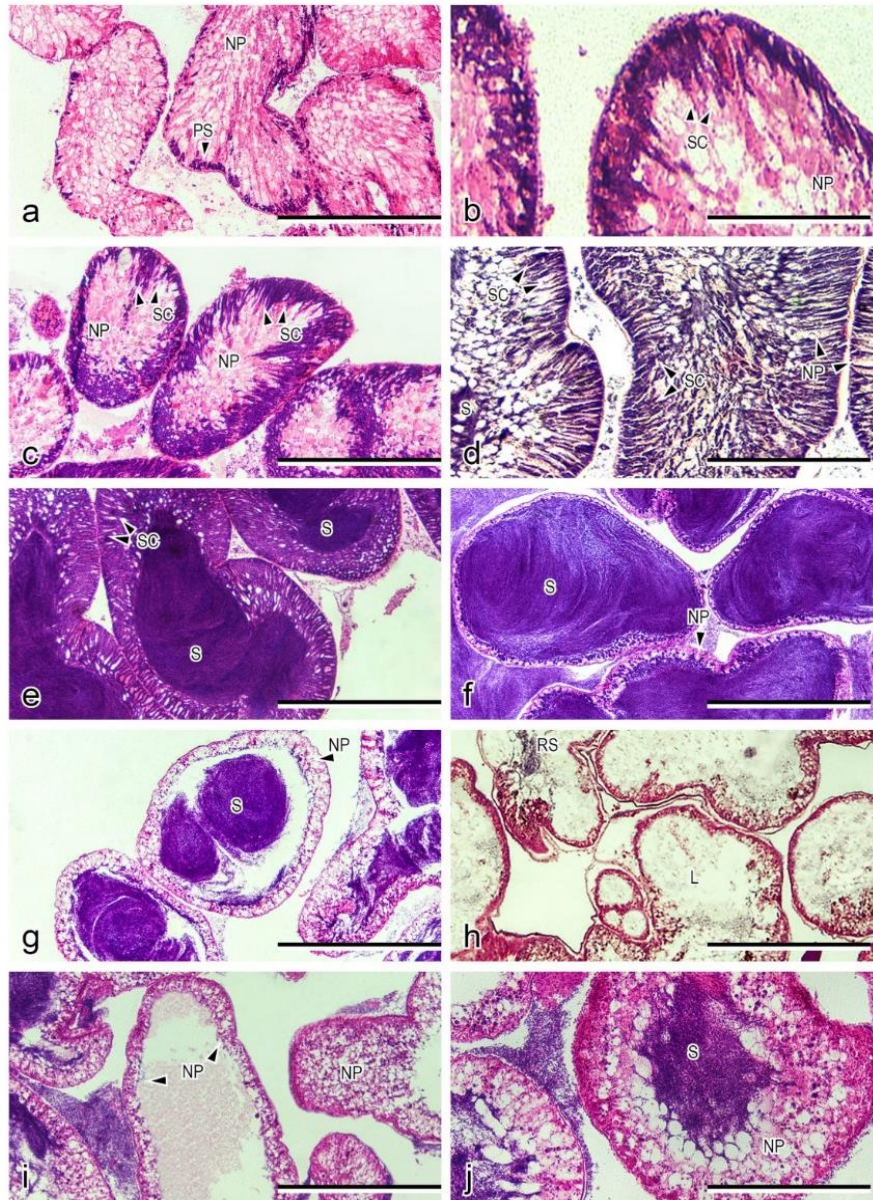


**Figure 9:** Gametogenic stages of *Centrostephanus rodgersii* shown as relative frequencies at a) St Helens 2003-05; b) St Helens 2014-15; c) Bicheno 2003-05; d) Mistaken Cape 2003-05; and e) The Lanterns 2003-05 (n=15 per sampling occasion across sites and study periods).



**Figure 10:** Histology of *Centrostephanus rodgersii* ovaries at all gametogenic stages; a) early-recovering ovary with lightly packed nutritive phagocytes; b) late-recovering, nutritive phagocytes dominate the lumen and pre-vitellogenic oocytes are developing in the germinal layer; c) Ovaries in the early growing phase displaying slightly lessened nutritive phagocytes density and pre- to early-vitellogenic oocytes around the periphery of the lumen; d) late growing ovaries with early and vitellogenic oocytes developing; e) early premature ovaries with oocytes beginning to take a central position within the lumen and a small amount of nutritive tissue between the germinal epithelium and vitellogenic oocytes; f) late premature ovary with mature oocytes in the centre of the lumen and limited nutritive phagocytes; g) mature ovaries with oocytes completely filling the lumen and no vitellogenic oocytes in the germinal layer; h) part-spawned ovary characterised by large spacing between mature oocytes; i) spent ovary with relict ova in the lumen and small amounts of nutritive phagocytes; j) part-spawned ovary beginning to recover with nutritive phagocytes surrounding residual ova. (NP = nutritive phagocytes; PO = pre-vitellogenic oocytes; EV = early-vitellogenic oocytes; VO = vitellogenic oocytes; O = oocytes; R = relict oocytes; scale bar = 250  $\mu$ m).





**Figure 11:** Histology of *Centrostephanus rodgersii* testes at all gametogenic stages; a) recovering testes with nutritive phagocytes dominating the lumen and small primary spermatocytes developing along the germinal epithelium; b) early growing testes with short spermatocyte columns beginning to develop, scale bar = 100  $\mu$ m; c) late growing testes showing elongated spermatocyte columns protruding to the centre of the lumen; d) early premature testes with large spermatocyte columns extending to the centre of the lumen and spermatozoa accumulating around the tips of columns; e) late premature testes with large spermatocyte columns projecting towards the centre of the lumen accumulating in a central mass; f) mature testes with spermatozoa completely filling the lumen and a reduced nutritive phagocyte layer; g) part-spawned testes with space between the acinal wall and central mass of spermatozoa due to spawning; h) spent testes with spermatozoa mostly vacated the lumen and only a small amount of relict spermatozoa remaining; i) early recovering testes with nutritive phagocytes beginning to build around the acinal wall; j) part-spawned testes in the early stage of recovery with nutritive phagocytes surrounding residual spermatozoa prior to the onset of phagocytosis. (NP = nutritive phagocytes; PS = primary spermatocytes; SC = spermatocyte columns; S = spermatozoa; L = lumen; scale bar = 250  $\mu$ m).

Image analysis of constructed histological sections showed mean proportional density of NP's (% area) within ovaries and testes to be highest in the early reproductive stages (i.e. 'recovering', 'growing', 'premature') before gametogenic differentiation (Table 1). This observation was consistent across both study periods and particularly evident from March to June inclusive. One-way ANOVA showed significant variation ( $P < 0.001$ ) of NP density between months within the 'recovering' and 'premature' reproductive stages across both study periods. However, there was no significant variation ( $P > 0.198$ ) of NP density within the 'growing' reproductive stage for both study periods indicating that NP density is stable across months while 'growing'.

Peak mean proportional density of NP's within the gonad lumen was 86% in May (2014) and observed during the 'premature' reproductive stage. NP's rapidly diminished to 51.3% the following month as mitotic proliferation of gonial cells began. During July, a proportion of samples collected had transitioned to 'mature' reproductive stage where mean NP density was recorded at 15.8% indicating a large reduction in NP's. All individuals in August (2014) were categorised as 'mature', 'part spawned' and 'spent' showing low mean NP density within gonads (Table 1). During this time, most individuals were either prepared to spawn with a large proportion of gametogenic cells or had recently spawned with mostly vacant gonads. After the major spawning period of August and September, gonads were mostly 'recovering' and NP's consistently increased in mean density until the following spawning season.

**Table 1:** Mean proportion (%) of NP's within gonads of *Centrostephanus rodgersii* throughout both study periods (2014-15 and 2003-05) at each reproductive stage.

(2014-15) Month	Reproductive Stage					
	Recovering	Growing	Premature	Mature	Part spawned	Spent
May	-	71.5	86.0	-	-	-
June	-	81.1	51.3	-	-	-
July	-	-	31.2	14.0	-	-
August	-	-	-	12.5	15.4	3.4
September	24.3	-	-	15.7	13.0	24.3
October	28.2	-	-	12.0	4.0	-
November	37.7	-	-	-	-	20.6
January	45.8	-	-	-	-	-
March	67.7	55.1	-	-	-	-
April	77.0	71.4	83.0	-	-	-
May	-	66.7	65.3	-	-	-
June	-	71.4	53.6	-	-	-
July	-	-	41.4	15.8	-	-
August	-	-	-	11.3	3.0	15.0
September	26.0	-	-	16.4	4.7	12.5
October	19.9	-	-	14.3	8.0	-

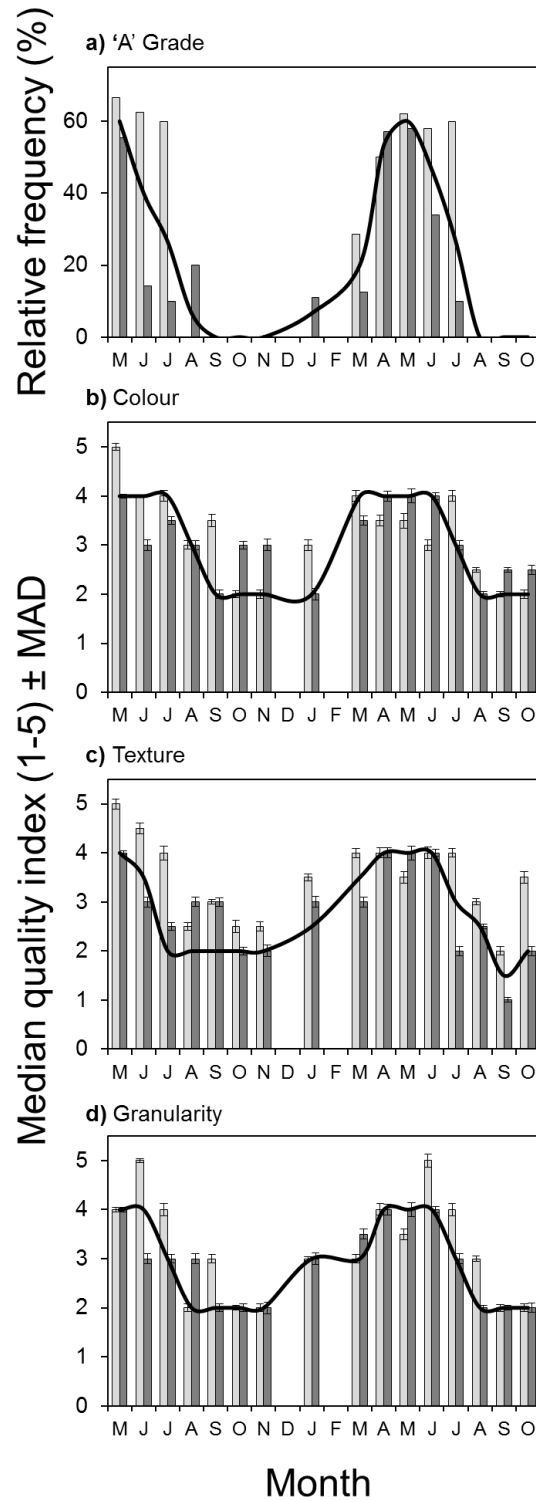
(2003-05) Month	Reproductive Stage					
	Recovering	Growing	Premature	Mature	Part spawned	Spent
November	33.0	-	-	-	-	-
February	62.4	-	-	-	-	17.1
March	65.5	80.5	78.0	-	-	-
April	-	78.7	-	-	-	-
June	-	70.0	58.0	-	-	-
August	-	-	53.0	12.0	7.8	-
October	-	-	-	-	-	-
December	29.8	-	-	-	-	18.8
April	85.0	78.5	-	-	-	10.0

#### 2.4.2 Seasonal variation of roe quality and effects of gender and nutritive phagocytes

Roe quality metrics across the 2014-15 study period showed similar and consistent trends, generally peaking during the same months each year. Colour, texture and granularity scored highest between March and June, then rapidly diminished through July, August and September (late winter and early spring) to the lowest recorded values of the study period. There were substantial differences in roe quality between genders, which was most evident from June to September during the later stages of

gametogenesis and spawning (Figure 12). Male *Centrostephanus rodgersii* scored higher roe quality indices compared to females during a majority of the study period. Gender significantly affected the five levels of texture ( $X^2 = 29.367$ ,  $df = 4$ ,  $P < 0.001$ ) and granularity ( $X^2 = 22.345$ ,  $df = 4$ ,  $P < 0.001$ ). Roe colour was not significantly affected by gender ( $X^2 = 9.648$ ,  $df = 4$ ,  $P = 0.345$ ).

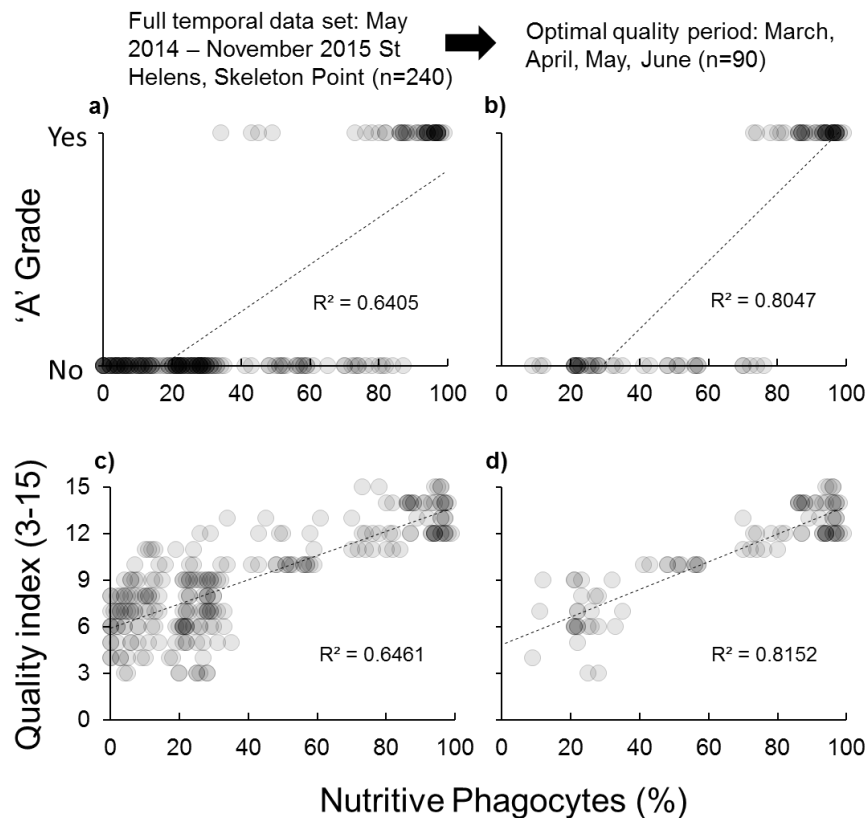
Logistic regression showed gender to be a significant contributing factor to 'A' grade roe ( $G^2 = 16.349$ ,  $P < 0.001$ ) and the odds ratio of collecting male individuals with 'A grade' roe was 1.23 times more likely (95% CI = 0.097, 1.711) when compared to females. Proportional 'A' grade peaked between April and June and consisted of between 40-60% of collected sea urchins (Figure 12a). After this period, proportional 'A' grade dramatically dropped and the performance between genders became more pronounced. Male and female gender ratios did not significantly deviate from 1:1 at St Helens 2014-15 ( $X^2 = 0.017$ ,  $df = 1$ ,  $P = 0.897$ ) nor did ratios deviate 1:1 across the 2003-05 sampling period ( $X^2 = 0.182$ ,  $df = 1$ ,  $P = 0.643$ ).



**Figure 12:** Seasonal variation of a) 'A grade' frequency b) colour c) texture d) granularity over the 2014-15 study period at St Helens (Skeleton Point) given as median values split between male (light grey bars) and female (dark grey bars)  $\pm$  median absolute deviation (MAD). Indices were estimated between 1-5 for individual roe quality metrics according to specific criteria. Solid line indicates the total median value for male and female for each roe quality metric and frequency 'A grade' (n=15 per month).



To examine the effect of NP's on 'A grade' categorically defined as 'success' (yes/no), and 'quality index' (combination score across roe quality metrics), sea urchins were assessed for relative proportion of NP's (% area) within the gonad lumen and relative roe quality scores. There was moderate correlation between NP's and roe quality indicating that higher proportions of NP's were positively affecting roe quality ('quality index' and 'A grade') across the full temporal data set (Figure 13a, c). However, the strength of this relationship was more pronounced when data focused on the peak roe quality period between April and June (Figure 13b, d). Logistic regression analysis across the full temporal data set showed a significant effect of NP's on 'A grade' ( $G^2 = 47.864$ ,  $P < 0.001$ ) and indicated that the odds ratio was 4.3 times more likely (95% CI = 1.647, 8.412) when NP's were in 'high' proportions. Interestingly, the interaction effect of NP's and gender and therefore the likelihood of sampling 'A grade' roe when *C. rodgersii* were male with a high proportion of NP's was significant ( $G^2 = 7.466$ ,  $P < 0.001$ ) and increased the odds ratio to a highly probable 9.67 times more likely (95% CI = 1.976, 58.316).



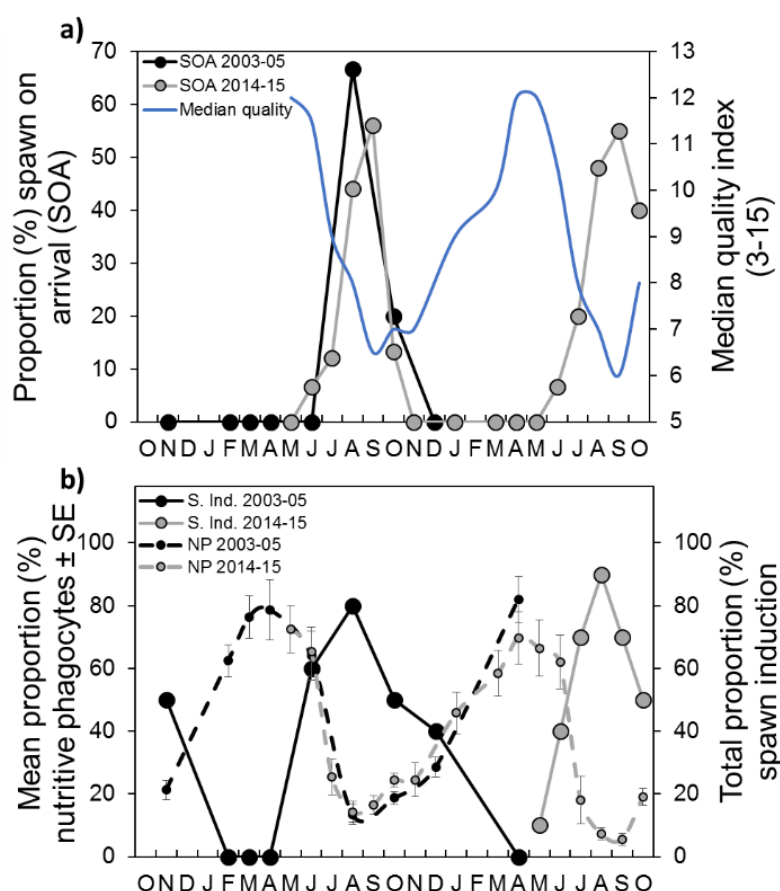
**Figure 13:** Relationship between nutritive phagocytes and roe quality ('A' grade and quality index) across the full temporal data set and peak roe quality period between April and June inclusive.

### 2.4.3 Temporal patterns and effects of spawning

There was temporal consistency across both study periods at St Helens for spawning on arrival (SOA) of *Centrostephanus rodgersii* after collections (simulated harvesting). First evidence of SOA during the 2014-15 study period was in June (6.67%) and further increased the following month (12%). SOA rapidly increased to 44% in August (2014-15) before a peak of 56% in September (Figure 14). Following the peak spawning period (August-September), SOA reduced to 13.3% in October, after which there were no observed SOA until the following season. This temporal pattern of SOA was consistent between both seasonal cycles of the 2014-15 study period. The 2003-05 study period showed a similar temporal pattern of SOA, albeit slightly earlier. Temporal median 'quality index' (sum of all individual roe quality metrics i.e. colour, texture, granularity) showed an inverse relationship to SOA and was highest in March-June. Median 'quality index' rapidly decreased through June and July as SOA

increased leading to the peak spawning period indicating that when SOA occurs in high proportions, roe quality is poor (Figure 14).

First evidence of spawning response from KCl injection during the seasonal reproductive cycle occurred in May, where the proportional rate of response was 60% and 10% for the 2003-05 and 2014-15 study periods respectively (Figure 14b). Spawning response from KCl injection rapidly increased to peak in August at 80% (2003-05) and 90% (2014-15). At this time, mean NP density within gonads was at the lowest recorded levels. This indicates that periods when KCl injection response is high, mean NP density (and roe quality) is likely to be low. Overall, SOA and spawning response to KCl injection followed a similar temporal pattern; however, KCl response occurred 1-2 months earlier as NP density and roe quality began to diminish.



**Figure 14:** Temporal patterns and effects of spawning at St Helens; a) spawning on arrival (SOA) for both study periods (2003-05 and 2014-15) and median quality index (2014-15) and; b) spawn induction using KCl injection (S. Ind.) and mean density of nutritive phagocytes (NP) across reproductive stages during the 2003-05 and 2014-15 study periods.

## 2.5 Discussion

### 2.5.1 Temporal and spatial reproductive periodicity

*Centrostephanus rodgersii* in Tasmania has a well-defined and predictable seasonal reproductive cycle. Sea urchins at all sites had winter/spring spawning periods with major gamete release beginning in August/September and relict presence of gametes through to October/November. While statistical analyses revealed evidence for slight differences in timing of GI between decades within St Helens, there was remarkable synchrony in overall patterns. Byrne et al. (1998) describe spawning activity beginning in June along the New South Wales coast, and King et al. (1994) detail spawning beginning July in Sydney, New South Wales. This is slightly earlier than Tasmanian populations (~one/two months), and applies to the onset of other gametogenic stages. While it is clear that gametogenic timing occurs later in the southern range-extension region of Tasmania relative to New South Wales, the mechanisms remain uncertain. It is not unexpected that there is evidence of a slight shift in the timing of gametogenesis in the more southern Tasmanian sites considering the sedentary nature of *C. rodgersii* (Flukes et al. 2012) and the potential for different environmental regimes and reproductive cues across such large spatial gradients (~1700 km). Pecorino et al. (2013) found that the timing of gametogenesis of *C. rodgersii* populations in a recently colonised area in northern New Zealand was highly synchronous with populations at similar latitudes in New South Wales. This suggests that change in environmental conditions, such as photoperiod or temperature (as postulated by Byrne et al 1998; Ling et al 2008) may play a role in the delay of gametogenesis in Tasmania.

Currently, there is no evidence that Tasmanian populations of *C. rodgersii* are self-recruiting given the poor larval development <12°C (Ling et al. 2008). Therefore, recruitment of larvae into Tasmania is highly variable and solely reliant on spawning populations in NSW where larvae is transported via the EAC. Delay in the major spawning period of *C. rodgersii* populations in Tasmania may increase the chances for successful larval development as sea surface temperatures begin to rise at the onset of spring

(Banks et al 2007). If the Tasmanian *C. rodgersii* fishery is reliant on larval recruitment from populations in NSW (Banks et al. 2010), and if the developing fishery were to expand, continuing reliable recruitment will be essential for the fishery to be sustainable into the future.

### **2.5.2 Roe quality of *Centrostephanus rodgersii***

Information on optimal harvest windows (HW's) and strategies to maximise quality has been lacking for the *Centrostephanus rodgersii* fishery in Tasmania and has limited its development to date. We observed that the presence of NP's significantly affected the likelihood of harvesting 'A' grade roe and therefore targeting periods in the reproductive cycle when NP's are most abundant will potentially increase the proportion of harvested 'A' grade roe. During both study periods (2003-05 and 2014-15), image analysis of histological gonad condition showed the highest mean NP proportional density was consistently represented by early reproductive stages (i.e. 'recovering', 'growing' and 'premature'), particularly between March and June. The timing of these reproductive stages was spatially and temporally consistent throughout decadal study periods suggesting that optimal HW's have been consistent for the last 10 years in Tasmania. Therefore, the consistency of *C. rodgersii* gametogenic periodicity observed in this study also suggests it is likely that defined HW's would persist into the future.

To a lesser degree, logistic regression showed gender to be a significant factor increasing the odds ratio of harvesting 'A' grade roe. Males and females were relatively equal in proportion of 'A' grade during the high roe quality period (March-June). However, the effect of gender became more pronounced in June and July when gametogenic differentiation was occurring, possibly due to females producing large oocytes. Phillips et al. (2009) observed a similar effect of gender in *Evechinus chloroticus* for sensory quality (i.e. taste, odour, texture and flavour) and concluded that maturing gametogenic cells were having an effect on gender separation of roe quality. Unfortunately from a commercial harvest point of view, sea urchins are sexually monomorphic and it is not possible for divers to target male *Centrostephanus rodgersii* by external features.

Overall, we propose that the commercial fishing season of *Centrostephanus rodgersii* in Tasmania for premium markets should plan to conclude in late May to early June to maximise the chances of harvesting 'A' grade quality roe and avoid a decline in quality. However, mean GI did not peak until June/July and therefore there is potentially an eight-week period (rapid gametogenesis) before spawning that recoverable roe product is a high proportion of total weight harvested. To maximise returns from this fishery, sea urchin processors and wholesales could potentially diversify markets to cater for this period of low quality/high volume roe. This could also provide a platform for marketing of lower grades harvested during the optimal HW. Results from this study indicate that a potential signal for transitioning from high quality markets to lesser quality/high volume markets is spawning response to KCl injection.

There is no defined commercial season for *Centrostephanus rodgersii* in Tasmania and harvesting usually continues until SOA occurs in a majority of the catch (August/September). By this stage, roe quality is reduced to its lowest point and is characterised by low proportions of NP's and large developed gametes within gonads in preparation for spawning. When SOA is observed in high proportions, suppliers will usually instruct commercial harvesters to cease operations. The results presented here demonstrate that SOA is a poor indicator for the conclusion of the commercial harvest season because roe quality is likely to be low in a majority of the catch. Spawning response to KCl injection may be a better option as echinoderms can respond to KCl injection when mature gametes are present (i.e. Cochran and Engelmann 1976; Morgan and Jangoux 2002). This study indicates that the rate of response to KCl injection will signal the relative proportion of NP's indicative of roe quality. Response to KCl injection first occurred in May, and rapidly increased as gametogenic cells developed. This period marked the start of declining roe quality through gametogenesis and we suggest that when spawning response to KCl injection is observed in high proportions, sea urchin processors need to alter supply strategies to not compromise premium markets by consigning inferior product.

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**Chapter 3: Environmental and biological drivers of high quality roe for the long-spined sea urchin (*Centrostephanus rodgersii*) in Tasmania**

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### 3.1 Abstract

The long-spined sea urchin fishery (*Centrostephanus rodgersii*) in Tasmania has been limited by high variability in roe quality which affects both price and processing cost yet the specific drivers remain mostly undefined. Predicting roe quality of sea urchins is inherently problematic due to the large range of factors that play a role and has been a major barrier to development of the fishery. Here we assess exogenous and endogenous factors affecting *C. rodgersii* roe quality using an ordinal logistic regression approach. Samples were collected from two different habitats including ‘kelp beds’ and ‘widespread barrens’ over an 18 month period between May 2014 and October 2015 and assessed for roe quality. Four distinct models were developed based on; the ability to directly observe variables during harvest operations, consider variables when planning harvest operations, manipulate variables post-harvest, and variables that are reflective of repeated harvest operations that can be tailored to maximise favourable conditions. Independent variables that had most influence on roe quality included; seasonality, habitat, age and size. Ordinal logistic regression analyses indicated that sea urchins collected between April and June (OR 7.85 95%CI 4.97, 10.36), aged between 7-20yrs (OR 14.34 95%CI 9.67, 28.46) and from habitats dominated by macroalgae (‘kelp’ OR 4.78 95% CI 1.99, 4.87) were the best performing levels in each factor suggesting that large improvements in roe quality can be made by tailoring harvest operations to accommodate these effects. Overall, it was found that a large proportion of sea urchins <100mm test diameter scored well (69.87%) for roe quality index (i.e. the sum of all roe quality metrics) indicating commercial harvest operations will maximise roe quality yield by targeting *C. rodgersii* individuals <100 mm TD within kelp dominated habitats and during the months of April, May and June in Tasmania.

### 3.2 Introduction

Sea urchins around the world are primarily harvested for their roe (reproductive organ) and either consumed domestically or exported to lucrative overseas markets. Considered a delicacy in many Asian cultures, sea urchin roe can attract significant economic returns for high quality product. Japan imports the majority of global sea urchin production (approximately 90%) and demand is highest for roe which



is firm, unbroken and bright yellow or orange (Sonu 2003). Prices for sea urchin product can range from USD\$10 to USD\$250/Kg for premium quality fresh chilled roe in premium Japanese markets (Wilens and Wessells 1997, Reynolds and Wilens 2000). As demand increased in the Japanese market, so did commercial fishing pressure on wild Japanese stocks. This resulted in overexploitation, serial depletion and in some cases complete collapse of certain Japanese sea urchin fisheries (Tegner and Dayton 1977, Lesser et al. 1998, Berkes et al. 2006). What transpired was the exploration of other potential sea urchin fisheries to identify species that can deliver high quality roe and consequently the development of sea urchin fisheries in North and South America were investigated. However, the development and operation of these fisheries remained largely unregulated which has led to an eventual decline in these fisheries also.

Australian sea urchin species have typically been regarded by overseas markets as poorer quality compared to species from Japan or the Americas (Mr Allen 2014, pers. comm). However, recent expansion of the Australian domestic market and new attempts at exporting has resulted in small yet consistent expansion of Australian sea urchin fisheries (Worthington and Blount 2003). Overall, commercial fishing for Australian sea urchins has been slow to develop due to variable roe quality and high processing costs (Blount and Worthington 2002). There are small established fisheries in south-eastern Australia where a majority of the commercial harvest has focused on *Heliocidaris* sp. although more recently attention has focused on *Centrostephanus rodgersii*, where the available biomass is considered to be significant (Andrew and Underwood 1989, Andrew and O'Neil 2000, Ling et al. 2009). In Australia, *C. rodgersii* is distributed throughout New South Wales (NSW), eastern Victoria, and as a consequence of an increasing East Australian Current (EAC), has undergone range-extension into eastern Tasmania (Ling et al. 2008, Johnson et al. 2011). This species has increased in abundance on Tasmania's east coast and now occurs in high "commercially harvestable" densities.

High densities of *Centrostephanus rodgersii* leads to intense herbivory which frequently causes widespread collapse from kelp beds to 'urchin barrens' devoid of foliose macroalgae (Lawrence 1957, Fletcher 1987, Andrew and Underwood 1989, 1993, Underwood et al. 1991, Ling 2008, reviewed by

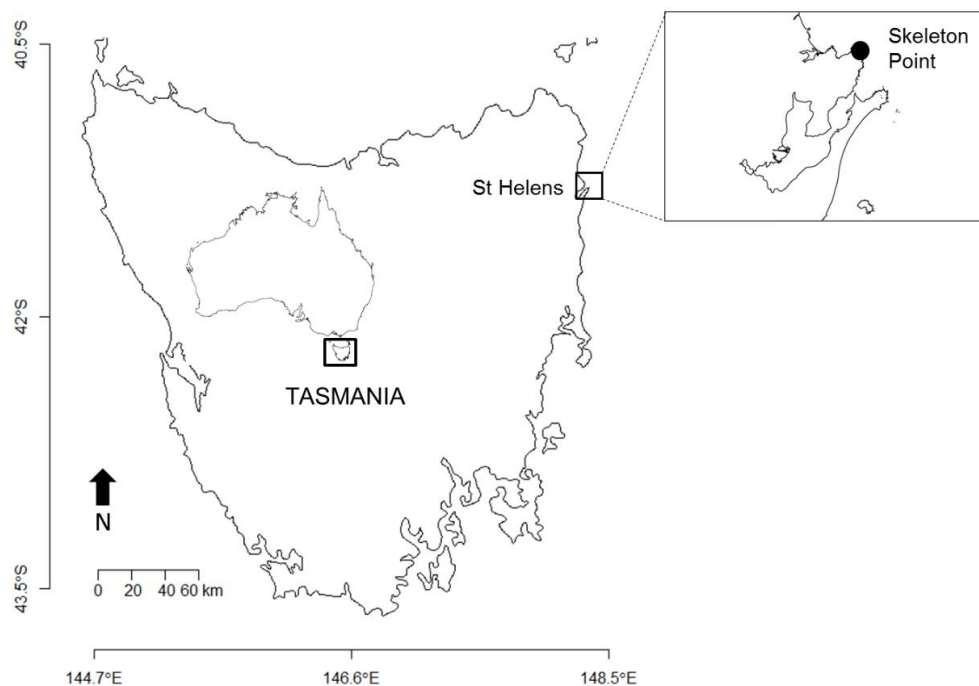
Ling et al. 2014). *C. rodgersii* can persist on barrens thus created by switching diet from kelp to less nutritious encrusting algae, slowing growth and impacting body and reproductive condition (Byrne et al. 1998, Ling & Johnson 2008). Along with ecological destruction has come economic opportunity as this species has presented a new fishery with significant potential for commercial exploitation. In 2009 commercial harvesting began, however, only small quantities have been harvested to date. Variable roe quality in catches has been the major constraint to this developing fishery and further understanding will assist in improving economic viability.

Roe quality of sea urchins is determined by market criteria based on physical condition and quality attributes (e.g. shape, size, texture and free of leaking fluids) of the reproductive organ (termed roe). Understanding the factors that drive reproduction and how they relate to roe quality of *Centrostephanus rodgersii* is required to optimise harvest efficiencies and improve quality of commercial catches. In this study we aim to identify contributing environmental and biological factors that relate to roe quality.

### **3.3 Methods**

#### **3.3.1 Sample collection**

Samples of *Centrostephanus rodgersii* were collected monthly from May 2014 to Nov 2015 from Skeleton Point, St Helens (41°14'54"S; 148°19'46"E) in north-east Tasmania (Figure 15). Samples were not collected during December 2014, February 2015 and November 2015 of the study period due to weather and logistics. Sample size was based on power analysis and calculated *a priori*. Where possible, specimens >80 mm were collected to reduce potential size related bias. All collections were performed using SCUBA from depths ranging 8-15m. Monthly samples were collected from two different habitat classifications; kelp beds (n=15) and extensive barrens (n=15) where habitats are typically indicative of high and low food availability respectively. Kelp bed habitats are typified by dense macroalgal assemblages, whereas extensive barrens are characterised by reef completely devoid of macroalgae and dominated by coralline algae. The transition zone from kelp beds to extensive barrens occurs at ~12m where healthy kelp bed habitat is found in the shallower margins.



**Figure 15:** Study site location for sampled *Centrostephanus rodgersii* in St Helens, from 15/05/2014 to 18/11/2015.

### 3.3.2 Morphometrics and Biometrics

Samples collected from field sites during 2014-15 were transported to the Institute for Marine and Antarctic Studies (IMAS) fish laboratory on the day of collection and processed the following morning. All sea urchins were stored at room temperature in lightly packed crates (fish bins). Test diameter (overall width of shell 'TD') and spine length using the longest apparent spine (methods defined in Ling and Johnson 2009) were measured using digital callipers ( $\pm 0.1$  mm). Total weight was measured by weighing whole animals to nearest 0.1g. To measure water loss at time of processing, incisions were made in the peristomial membrane to drain coelomic fluid and again weigh the specimen with all components (minus total coelomic fluid). One jaw piece was excised from the Aristotles' lantern and placed in sodium hypochlorite for 48hrs to remove residual tissue prior to taking jaw length measurement ( $\pm 0.1$ mm) for age estimation (detailed below). Gut composition was qualitatively

assessed for percentage (%) of total macroalgae and coralline algae. Gonads were excised and weighed and gonad somatic index (GI) was calculated as a percent body weight as follows:

$$GI(\%) = \frac{\text{Total gonad weight (g)}}{\text{Total body weight (g)}} \times 100$$

### **3.3.3 Age**

Age of sea urchins was estimated using the field calibrated growth increment model developed by Ling and Johnson 2009 for kelp and barrens. The model was developed from tagging data and used an inverse logistic function of annual jaw length growth increment verses initial size at tagging. Jaw lengths were measured with knife-edge callipers from the upper margin of the jaw to the point where the tooth structure begins to protrude. Frequency distributions of age and size were conducted using pair-wise Kolmogorov-Smirnov tests between habitat types.

### **3.3.4 Roe quality scoring**

Colour, texture and granularity formed the basis of roe quality criteria and each metric was qualitatively assessed by allocating a rank score of 1-5 with 5 being the maximum and 1 being the minimum score for each category. Standard commercial colour cards (DPIPWE sea urchin catch report docket book) were used to assess colour where 1 = dark brown, through to 5 = bright yellow. Texture was assessed and scaled using commercial criteria based on the degree to which roe maintained its shape and definition, where 1 = sloppy, and 5 = firm. Granularity was assessed according to granular surface definition and based on the qualitative size of gonad lumen tubules (1-5). Here, 1 = a rough gonad with large tubules, and 5 = a smooth gonad with small tubules. An overall roe quality metric of 'quality index' was determined by summing the scores for colour, texture and granularity (combination score between 3-15 because 1 is the minimum score for each metric). Individual sea urchins were categorised into 'A' grade and 'B' grade where 'A' grade individuals required a categorical score of 4 or above in all roe quality criteria as per commercial standards.

### 3.3.5 Data analyses

All data analyses were conducted using R 3.0.3 (R Core Team, 2014). Two-way ANOVA was used to evaluate differences in GI and average ‘A’ grade yield per individual between habitats. Four distinct ordinal logistic regression models were developed (see below) to assess the effects of independent variables on roe quality metrics using the ‘polr’ function in the statistical package R. Models were based on; (1) factors a commercial harvest operation can control (i.e. when to harvest and from which habitat); (2) parameters a commercial diver can identify during harvesting (i.e. morphological indicators); (3) parameters commercial operations can control post harvesting; (4) parameters that cannot be controlled but inform harvest decisions. Initial collinearity matrices were constructed for relevant models and no omissions were required (i.e. correlations  $\geq 0.6$ ). Model 1 was developed using independent variables seasonality (Phillips et al. 2010a) and habitat (Blount and Worthington 2002). The fixed factor of seasonality was categorised into four levels describing annual calendar months (i.e. Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec) throughout the study period and overlap in monthly data were pooled into relevant categories. The fixed factor habitat was categorised into levels of ‘kelp’ (healthy intact macroalgal assemblages), ‘extensive barrens’ (substrate devoid of macroalgae). The model is illustrated below;

$$(*\textbf{Model 1}) \text{ roe quality } (y_i) = \beta_0 + (\text{Habitat}) + (\text{Seasonality}) + \varepsilon_i$$

Where;  $y_i$  is the roe quality metric (i.e. colour, texture, granularity or quality index),  $\beta_0$  is a vector of the predictor variable, Habitat = (kelp, barrens), Age = (0-20yrs, 21-40yrs, 41-60yrs, and >61yrs), Seasonality = (Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec), and  $\varepsilon_i$  is the error term.

Model 2 used continuous independent morphological predictor variables that commercial harvesters can potentially use to identify sea urchins with high quality roe. These included; test diameter (size/age correlations *see* Ling & Johnson 2009) and spine length (as a function of habitat Ling & Johnson. 2009) and is illustrated below;

$$(*\textbf{Model 2}) \text{ roe quality } (y_i) = \beta_0 + (\text{TD}) + (\text{SL}) + \varepsilon_i$$

Where;  $y_i$  is the roe quality metric (i.e. colour, texture, granularity or quality index),  $\beta_0$  is a vector of the predictor variable, TD = Test Diameter, SL = Spine Length, and  $\varepsilon_i$  is the error term.

Model 3 used continuous independent predictor variables that can potentially be manipulated to suit optimal roe quality post-harvest operations. These included water loss and time out of water which can be optimised by adjustments to handling and processing regimes. The model is illustrated below;

$$(*\text{Model 3}) \text{ roe quality } (y_i) = \beta_0 + (\text{WL}) + (\text{TOOW}) + \varepsilon_i$$

Where;  $y_i$  is the roe quality metric (i.e. colour, texture, granularity or quality index),  $\beta_0$  is a vector of the predictor variable, WL = Water Loss, TOOW = Time Out Of Water, and  $\varepsilon_i$  is the error term.

Model 4 used independent variables that are not directly observable during immediate harvest operations although can potentially inform strategies for harvesting high quality roe. The random effect of age (*see* Agatsuma et al. 2005), was categorised into four levels (i.e. 7-20yrs, 21-40yrs, 41-60yrs, and >61yrs) based on individual age estimates and gut composition (nutrient formulation on gonad condition *i.e.* de Jong-Westman et al. 1995, Meidal and Scheibling 1998) was categorised into percentage kelp in gut content (0%-25%, 26%-50%, 51%-75% and 76%-100%). The model is illustrated below;

$$(*\text{Model 4}) \text{ roe quality } (y_i) = \beta_0 + (\text{Age}) + (\text{Kelp in gut}) + \varepsilon_i$$

Where;  $y_i$  is the roe quality metric (i.e. colour, texture, granularity or quality index),  $\beta_0$  is a vector of the predictor variable, WL = Water Loss, TOOW = Time Out Of Water, and  $\varepsilon_i$  is the error term.

Preliminary data analyses showed that the worst performing levels for each independent categorical variable was; (model 1) 'Oct-Dec', 'barrens', and (model 4) >61yrs, 0%-25% kelp in gut content. Therefore, these levels for each independent categorical variable were chosen to be the baseline in ordinal logistic regression analyses from which odds ratios were constructed from exponentiated

coefficients and confidence intervals. The model was run separately for each roe quality response variable (colour, texture, granularity and quality index). Overall, the appropriateness of all models was assessed by testing the proportional odds assumption by regressing the dependent variable (roe quality metric) against independent variables across cut-points one at a time and comparing coefficients with the saturated model output. There were no difference in the coefficients therefore the null hypothesis was accepted and the proportional odds assumption was not violated.

### **3.3.6 Size frequency of commercial catch**

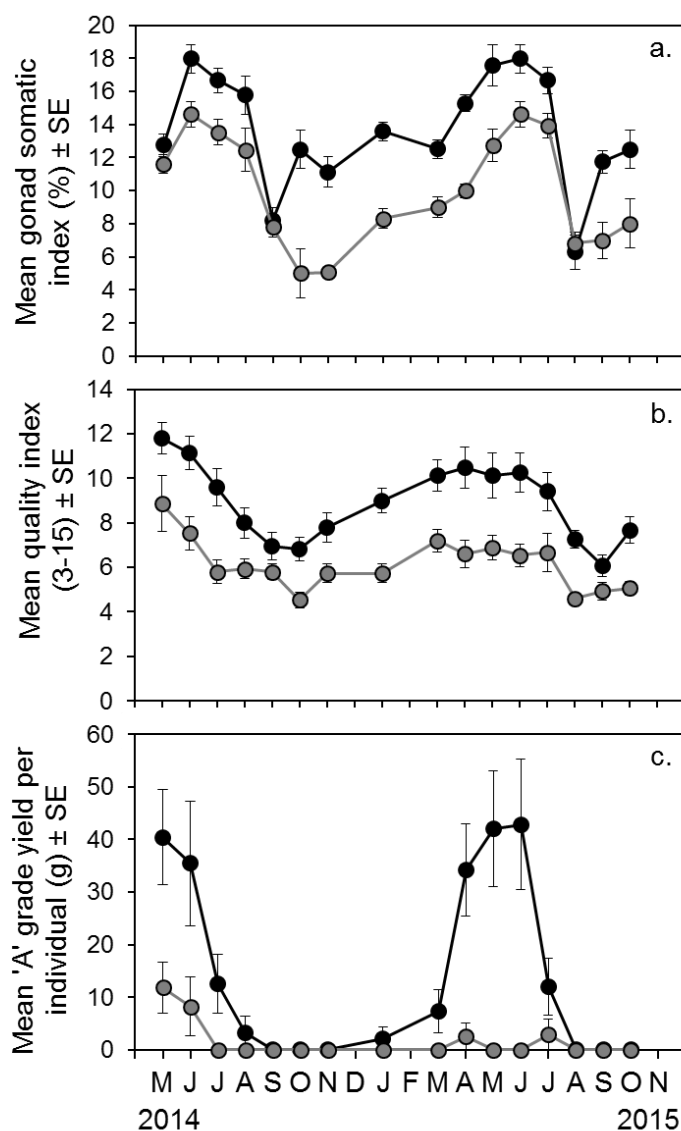
Commercially caught *Centrostephanus rodgersii* were measured for size frequency using digital callipers across the TD of the sea urchin excluding spines. Sea urchins were harvested on 20 July 2015 directly adjacent to study sites at Skeleton Point (41°14'54.48"S; 148°19'45.21"E), St Helens. All commercial catch samples were harvested by a commercial operator using typical commercial techniques in the kelp habitat, depth profile (~11m) and size distribution of catch (>80mm and <120mm). These data were included in Kolmogorov-Smirnov tests of size frequency distributions against habitat types.

## **3.4 Results**

### **3.4.1 Temporal reproduction and roe quality across habitat types**

Mean GI showed similar seasonal variation across habitat types with peaks occurring in June/July. A rapid decrease in GI was observed through August/September and was consistent in both seasonal cycles of 2014 and 2015 (Figure 16). The 'kelp' habitat consistently produced the largest GI across the study period, particularly from December to June. Two-way ANOVA indicated significant GI variation within ( $F = 4.68$ ,  $P = 0.03$ ) and between habitat types ( $F = 29.02$ ,  $P < 0.0001$ ) at each month across the temporal dataset. Mean roe quality index per month (sum of scored roe quality metrics per individual) showed a similar pattern to GI across habitats, although a rapid decrease in roe quality was observed approximately 1-2 months prior to a decrease in GI. Quality across habitats was observed to peak between the months of March to June (Figure 16). The 'kelp' habitat produced the highest average

quality index across the study period peaking between March and June. Individuals categorised as ‘A’ grade were averaged by GI to calculate the yield of ‘A’ grade roe. Mean ‘A’ grade yield per individual was largest between the months of March and June (Figure 16) and kelp beds outperformed barren habitat type for ‘A’ grade yield.



**Figure 16:** Temporal reproduction and roe quality patterns between 2014 and 2015 for habitats of: ‘kelp’ —●— (n=240) and ‘barrens’ -●- (n=240). a) Mean seasonal patterns in gonad somatic index. b) Mean quality sum index (sum of colour, texture and granularity indices). c) Mean ‘A’ grade yield per individual is the amount (g) of roe if individual is categorised as ‘A’ grade.



### 3.4.2 Morphometric, biometric and environmental predictors of roe quality

Model 1 showed that specific levels within each independent variable of seasonality and habitat were highly significant for all roe quality metrics (Table 2). Seasonality categories ‘Jan-March’ and ‘Apr-Jun’ significantly ( $P<0.01$ ) affected the odds ratio of harvesting higher quality roe. The largest effect of seasonality was for the combination of all roe quality criteria ‘quality index’ and was found to be highest during the months of ‘Apr-Jun’ where the odds of harvesting high quality roe was a highly probable 7.84 (95% CI 4.97, 10.36) times greater if other variables in the model are held constant. The ‘kelp’ habitat significantly ( $P<0.01$ ) affected the odds ratio and showed the strongest likelihood of encountering high quality roe. Texture was most influenced by ‘kelp’ habitat increasing the odds ratio to 5.83 times greater (95%CI 1.98, 4.61). This trend was consistent across all roe quality metrics for habitat (Table 2).

**Table 2:** Ordinal logistic regression results (n=480) for developed models. Model numbers are listed with asterisk (\*) in bold and the dependent and independent variables are indicated either side. Independent variables were regressed against dependent ordinal roe quality criteria including; colour, texture, granularity and the combination score of quality index.



	Model No.	Independent Variable		Coef.	Std. Error	P-value	Odds Ratio	2.5%	95%
<b>COLOUR</b>	<b>*1</b> AIC=2023.47, Res. Dev.=1897.34	<b>Seasonality</b>	<i>Jan-Mar</i>	1.22	0.29	<0.01	2.34	2.46	3.97
			<i>Apr-Jun</i>	1.87	0.23	<0.01	5.31	3.21	7.84
			<i>Jul-Sep</i>	0.29	0.19	0.08	1.87	0.48	1.56
			<i>Oct-Dec</i>	-	-	-	-	-	-
		<b>Habitat</b>	<i>Kelp</i>	1.41	0.17	<0.01	4.51	2.37	5.12
			<i>Barrens</i>	-	-	-	-	-	-
	<b>*2</b> AIC=597.13, Res. Dev.=549.34	<b>Test diameter</b>		-0.13	0.02	<0.01	1.31	0.94	1.03
		<b>Spine length</b>		-0.03	0.01	0.34	1.13	0.96	1.06
	<b>*3</b> AIC=689, Res. Dev.=597.12	<b>Water loss</b>		-0.36	0.03	0.04	1.64	0.92	0.98
		<b>Time out of water</b>		-0.46	0.01	0.03	1.72	0.91	0.99
	<b>*4</b> AIC=2946.67, Res. Dev.=2831.68	<b>Age</b>	<i>7-20yrs</i>	3.6	0.41	<0.01	8.37	4.97	16.37
			<i>21-40yrs</i>	1.6	0.36	<0.01	3.46	2.13	6.81
			<i>41-60yrs</i>	-0.64	0.38	0.34	0.69	0.69	1.76
			<i>&gt;61yrs</i>	-	-	-	-	-	-
		<b>Kelp in gut</b>	<i>0-25%</i>	-	-	-	-	-	-
			<i>26-50%</i>	-0.12	0.21	0.12	1.06	1.46	3.46
			<i>51-75%</i>	0.64	0.18	0.07	1.37	1.98	3.87
			<i>76-100%</i>	1.23	0.19	<0.01	3.21	2.06	4.97
<b>TEXTURE</b>	<b>*1</b> AIC=2221.47, Res. Dev.=2012.34	<b>Seasonality</b>	<i>Jan-Mar</i>	1.36	0.23	<0.01	2.87	2.37	3.81
			<i>Apr-Jun</i>	1.82	0.19	<0.01	6.34	3.79	7.91
			<i>Jul-Sep</i>	0.31	0.17	0.12	1.76	0.64	1.67
			<i>Oct-Dec</i>	-	-	-	-	-	-
		<b>Habitat</b>	<i>Kelp</i>	1.81	0.18	<0.01	5.83	1.98	4.61
			<i>Barrens</i>	-	-	-	-	-	-
	<b>*2</b> AIC=567.13, Res. Dev.=529.34	<b>Test diameter</b>		-0.11	0.01	<0.01	1.21	0.91	1.01
		<b>Spine length</b>		-0.34	0.01	0.23	1.15	0.99	1.11
	<b>*3</b>	<b>Water loss</b>		-0.61	0.01	0.02	1.19	0.93	0.99

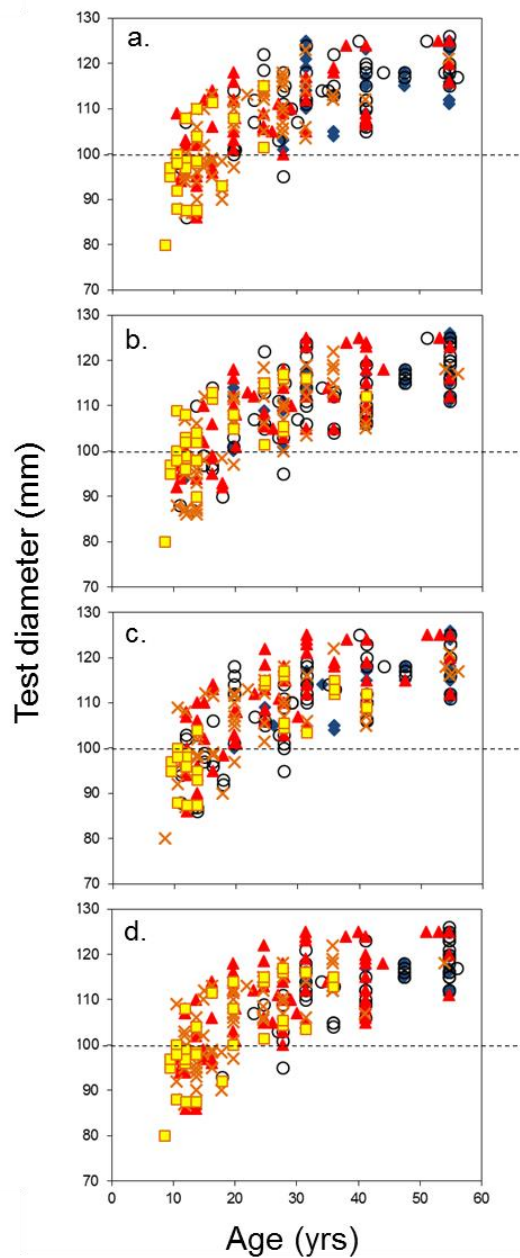
GRANULARITY	AIC=750.46, Res. Dev.=681.37		Time out of water	-1.21	0.02	0.01	2.1	0.95	1.13		
	*4	Age	7-20yrs	3.78	0.46	<0.01	7.23	5.67	17.56		
			21-40yrs	1.84	0.4	<0.01	4.13	2.87	6.97		
			41-60yrs	-0.34	0.42	0.22	0.78	0.84	1.91		
			>61yrs	-	-	-	-	-	-		
	AIC=2674.64, Res. Dev.=2413.78	Kelp in gut	0-25%	-	-	-	-	-	-		
			26-50%	-0.46	0.24	0.22	0.81	0.98	3.37		
			51-75%	0.98	0.21	0.08	1.24	1.23	3.46		
			76-100%	1.67	0.22	<0.01	2.97	1.87	4.87		
	AIC=2166.7, Res. Dev.=1978.33	*1	Seasonality	Jan-Mar	1.12	0.22	<0.01	2.12	2.27	3.79	
				Apr-Jun	2.13	0.20	<0.01	5.94	3.41	7.61	
				Jul-Sep	0.49	0.21	0.09	1.98	0.56	1.03	
				Oct-Dec	-	-	-	-	-	-	
		Habitat	Kelp	1.36	0.19	<0.01	5.31	1.84	4.44		
			Barrens	-	-	-	-	-	-		
			*2	Test diameter		-0.26	0.02	0.02	1.07	0.96	1.08
					Spine length	-0.46	0.01	0.29	0.76	0.97	1.22
	AIC=620.13, Res. Dev.=576.84	*3	Water loss	-0.71	0.01	0.03	1.46	0.94	1.13		
			Time out of water	-1.64	0.01	0.02	1.31	0.97	1.21		
	AIC=2433.19, Res. Dev.=2234.67	*4	Age	7-20yrs	2.69	0.37	<0.01	5.39	6.37	18.36	
				21-40yrs	1.06	0.34	<0.01	4.89	3.12	7.21	
				41-60yrs	-0.13	0.36	0.29	1.12	0.91	2.13	
				>61yrs	-	-	-	-	-	-	
		Kelp in gut	0-25%	-	-	-	-	-	-		
			26-50%	-0.79	0.29	0.28	0.76	0.99	3.47		
			51-75%	1.32	0.22	0.13	1.23	1.76	3.52		
			76-100%	1.78	0.26	<0.01	1.67	1.91	4.91		
AIC=3167.13, Res. Dev.=2897.14			*1	Seasonality	Jan-Mar	1.67	0.34	<0.01	4.12	3.39	5.12
					Apr-Jun	2.78	0.30	<0.01	7.84	4.97	10.36
	Jul-Sep	0.45			0.32	0.06	1.45	0.78	1.29		
	Oct-Dec	-			-	-	-	-	-		
	Habitat	Kelp	1.56	0.21	<0.01	4.78	1.99	4.87			
		Barrens	-	-	-	-	-	-			
		*2	Test diameter		-0.37	0.01	0.01	1.46	0.9	1.12	
				Spine length	-0.78	0.02	0.13	1.12	0.99	1.29	
	AIC=710.98, Res. Dev.=651.78	*3	Water loss	-0.69	0.01	0.02	1.37	0.95	1.19		
			Time out of water	-1.98	0.02	0.01	1.97	0.98	1.39		
AIC=3214.34, Res. Dev.=3146.25	*4	Age	7-20yrs	2.98	0.29	<0.01	14.34	9.67	28.46		
			21-40yrs	1.34	0.27	<0.01	4.78	4.69	9.13		
			41-60yrs	-0.89	0.28	0.34	1.78	0.98	2.67		
			>61yrs	-	-	-	-	-	-		
	Kelp in gut	0-25%	-	-	-	-	-	-			
		26-50%	-0.64	0.27	0.22	1.26	1.64	3.64			
		51-75%	1.46	0.22	0.09	1.12	2.1	4.13			
		76-100%	1.98	0.26	<0.01	3.3	2.15	5.64			

Model 2 showed varied effects on roe quality where ‘test diameter’ was significant ( $P<0.01$ ) across all roe quality metrics and ‘spine length’ was not ( $P>0.05$ ). The magnitude of odds ratio’s and coefficients for ‘test diameter’ and ‘spine length’ was low across all roe quality metrics (Table 2), and indicated a negative effect for every unit increase. Independent variables in model 3 showed limited effects on roe quality. The effects of ‘water loss’ and ‘time out of water’ were found to be significant in the model however the coefficients indicated little influence of these variables. Interestingly, the most significant effect in model 3 was ‘water loss’ on ‘quality index’ which increased the odds ratio to 1.97 (95% CI 0.98, 1.39).

Model 4 indicated significant effects of ‘age’ and ‘kelp in gut’ at certain levels with each variable. Age categories ‘7-20yrs’ and ‘21-40yrs’ were found to be highly significant ( $P<0.01$ ) for all roe quality criteria. Odds ratios of encountering high quality roe in these year categories was  $>5.39:1$  across all metrics. Year categories 41-60yrs and  $>61$ yrs were not significant in the model. The best performing ‘age’ category was 7-21yrs, where odds ratios ranged between 5.39:1 (95%CI 6.37, 18.36) for granularity and 14.34:1 (95%CI 9.67, 28.46) for overall quality index indicating that it is highly likely that superior roe quality can be harvested within this age bracket. Only one level within the independent variable ‘kelp in gut’ (i.e. 76%-100%) was shown to have a significant effect on roe quality and the odds ratio ranged from 1.67 (95%CI 1.91, 4.91) for texture, to a highly probable 3.3 (95%CI 2.15, 5.64) for quality index (i.e. sum of all quality metrics).

### **3.4.3 Age, test diameter and roe quality**

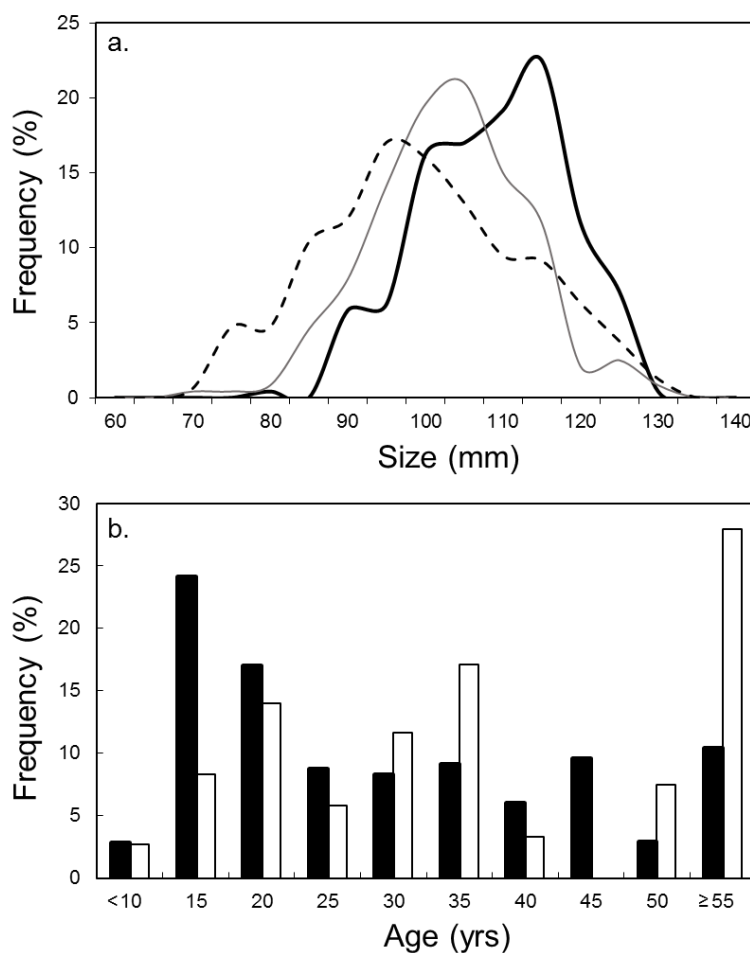
Ordinal logistic regression showed ‘age’ and ‘test diameter’ were significantly affecting roe quality throughout the study period. To assess the effect of these variables in the kelp habitat, data points from barrens habitat were removed and scatterplots constructed. The effect of ‘age’ and ‘test diameter’ on roe quality was further examined for sea urchins that had high independent roe quality scores (4 or 5) and were small in test diameter. It was found that the total proportion of sea urchins below 100mm TD that scored 4 or 5 for colour was 68%, 57% were below 100mm for texture and 53% were below 100mm for granularity. However, the largest proportion of high performing sea urchins  $<100$ mm that scored well was the combination category ‘quality index’ which resulted in a total of 69% individuals scoring  $\geq 10$  (top categorical scores i.e. ‘’ or ‘’).



**Figure 17:** Age and size effects on roe quality metrics in the kelp habitat. All metrics were assessed from 1-5 (  $\blacklozenge$  = 1;  $\blacktriangle$  = 2;  $\circ$  = 3;  $\times$  = 4;  $\blacksquare$  = 5) except roe quality index which scored between 3-15 (  $\blacklozenge$  = 3;  $\blacktriangle$  = 4-6;  $\circ$  = 7-9;  $\times$  = 10-12;  $\blacksquare$  = 13-15) where; a) = colour, b) = texture, c) = granularity and d. = quality index. (n=240) for each quality metric. Dotted line denotes the upper size limit (100mm).

### 3.4.4 Size and demographic distributions between habitats

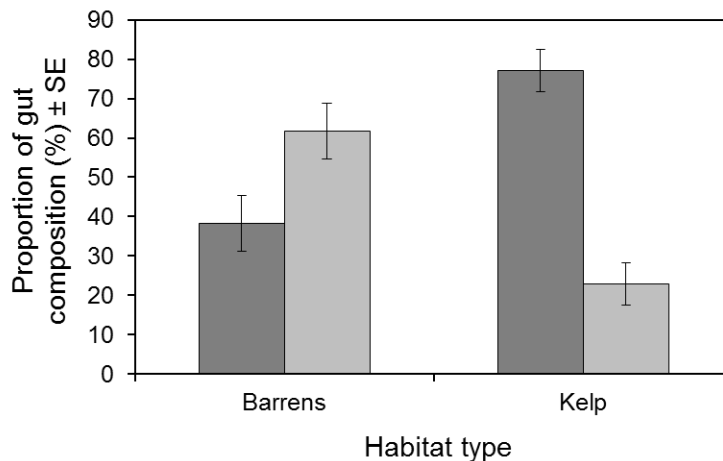
Size and age distributions were significantly different between habitat types for pair-wise Kolmogorov-Smirnov comparisons (i.e. minimum  $P < 0.0001$ ). Largest specimens were encountered in the kelp bed habitat where a majority of the individuals collected had test diameters between 100-120 mm, (Figure 18a). Age showed an inverse relationship between habitats, where the oldest individuals sampled were from barrens habitat and the youngest individuals were from the kelp habitat. Almost half the individuals sampled from kelp habitat (45.5%) were below 25 years of age, whereas only 24.5% of individuals sampled from the barrens habitat were below 25 years of age (Figure 18b).



**Figure 18:** Where; a. Size frequency distributions across habitats where; kelp = ‘thick’ black line; barrens = grey line; commercial catch = dashed line measured 4/6/2015 (n=120). b) Age frequency distributions of *Centrostephanus rodgersii* in kelp and barrens habitat types from structured monthly sampling. Intact kelp beds n=240 (black bars), extensive barrens n=240 (white bars).

### 3.4.5 Gut content and habitat

Gut content was averaged across the study period for percentage macroalgae and coralline algae. Macroalgae was found to be the highest proportion of gut content in kelp beds and encrusting coralline algae was in the highest proportion in extensive barrens (Figure 19). Individuals from kelp habitats averaged 76% macroalgae. However, individuals from barrens habitat averaged 38% kelp in gut content.



**Figure 19:** Average proportion gut content of macroalgae (dark shaded bars) and encrusting coralline algae (light shaded bars) per habitat type; kelp beds (n=240), extensive barrens (n=240).

## 3.5 Discussion

### 3.5.1 Roe quality and strength of predictor variables

Roe quality of *Centrostephanus rodgersii* was variable throughout the study period and within monthly sample periods which reflects the problem for commercial operators. The models developed to describe factors affecting roe quality found relationships between dependent and independent variables to be strongest for seasonality, habitat and age. In model 1, seasonality was shown to have a strong effect on roe quality where the best performing months were between 'April–June', closely followed by 'January–March'. The remaining two periods of 'July–September' and 'October–December' demonstrated very poor roe quality across all metrics. Prior to gametogenic differentiation and peak spawning, nutritive phagocytes (NP's) are most dense as these are the nutrient stores that contain all necessary lipids and

glycogen required for reproductive development. For *C. rodgersii*, rapid gametogenesis begins in late June through to August then spawning is initiated (King et al. 1994, Pecorino et al. 2013). After this time, the reproductive state is either spent or very early recovering as NP's begin to develop within the gonad. In the current study, this period was found to have poor roe quality when compared to the other periods in the calendar year.

Habitat was also found to be a highly significant contributor to roe quality where the best performing factor was 'kelp'. Kelp habitats are dominated by diverse macroalgal assemblages providing a wide range of nutrient composition and surplus availability. Nutritional availability (Garrido and Barber 2001, Dodge and Edwards 2012) and composition (Larson et al. 1980, de Jong-Westman et al. 1995, Fernandez and Boudouresque 2000) have been shown to affect gonad development and it is likely that kelp habitats are supplying the required nutrients for optimal gonad growth and consequent roe quality in *Centrostephanus rodgersii*. Interestingly gut composition (proportion macroalgae in gut) did not have a significant effect on roe quality except from the factor '76%-100%'. This suggests that high roe quality requires a large proportion of kelp in the gut which is indicative of kelp bed habitats.

Age was found to be a significant contributor to roe quality. The best performing age factors were '7-20yrs', closely followed by '21-40yrs'. The remaining age factors '41-60yrs' and '>61yrs' were not significant in the model and indicated diminishing roe quality with increasing age. Overall, younger sea urchins were found to produce small bright yellow roe with tightly packed tubules (fine grain) and maintained a solid shape which is highly desired by export markets (Reynolds and Wilen 2000). In a study by Agatsuma et al. (2005), older sea urchins were found to cause brown colouration in *Strongylocentrotus nudus* which can taint premium roe and drastically reduce economic returns. The preferred colour gonads have also been found in small *Centrostephanus rodgersii* with large gonads in the home range of New South Wales (Blount and Worthington 2002). This study indicated that high quality sea urchins were young and from macroalgal dominated habitats.

Test diameter was also found to significantly affect all roe quality metrics indicating that it is possible to target certain size classes to maximise the likelihood of harvesting younger individuals and high

quality roe. In general, sea urchins rely solely on their gonad for energy storage (Hammer et al. 2006; Hughes et al. 2006) and it is possible that younger sea urchins are putting more energy into somatic growth rather than reproductive output which is resulting in better market characteristics. Conversely, older sea urchins generally produced large dark or discoloured gonads with large gonadal tubules which are not classified as high quality by overseas markets.

There were significant differences in size distribution between habitats where the largest individuals were sampled in the 'kelp' habitat. Only a small proportion of individuals from the 'kelp' habitat were below 100mm (26.25%) yet a high proportion of these individuals displayed high quality roe. In contrast, the 'barrens' habitat had a high proportion of individuals that were <100mm, however the roe quality was poor. This pattern may have skewed the regression results and reduced the magnitude of effect for 'test diameter', adding further evidence that it is possible to target small individuals from kelp habitats to return high roe quality.

Spine length was found to have no significant interactions with roe quality. However, a study by Ling and Johnson 2009 has shown that spine length is highly reflective of habitat where shorter spined sea urchins are predominately found in kelp habitats due to the sweeping motion of macroalgae in exposed environments. Although there was no significant effect on roe quality for spine length in the current study, it may be possible to target shorter spine individuals that are reflective of residing in kelp habitat.

'Water loss' and 'time out of water' were found to be significant in model 3 across all roe quality metrics although the magnitude of the effect was relatively small when compared to other independent variables. Internal coelomic fluid may be acting as a protective agent and any reductions in this fluid may be resulting in oxidation of roe and diminishing quality characteristics. Generally when sea urchins are transferred from dive catch bags to crates (fish bins) there is a certain degree of squashing to fit in the crate. This may be resulting in spines and sharp edges piercing the peristomial membrane and therefore allowing coelomic fluid to escape the internal cavity of the sea urchin. Potentially more care could be given when packing sea urchins into crates which may minimise water loss and improve roe quality of



roe. Also, minimising the time sea urchins spend out of water may improve overall quality and can be achieved by simply organising fishing and processing to occur in coordination.

### **3.5.2 Reproduction and roe quality across habitats**

Gonad index showed a highly seasonal pattern across habitats peaking in the months of June and July as previously reported for the native home range in NSW (King et al. 1994, Byrne et al. 1998) and in Tasmania (Ling et al. 2008). There were significant differences in GI between habitats with consistently lower GI values found in the ‘barrens’ habitat, presumably due to limited food. On average, sea urchins and GI’s were larger in the ‘kelp’ habitats indicating that it is possible to harvest more recoverable product per individual in this habitat. As a consequence, ‘A’ grade yield per individual is also higher in kelp habitats increasing profitability and efficiency of harvest operations if a high proportion of individuals are harvested from this habitat. However, markets tend to prefer smaller sized gonads and therefore higher prices may be paid for smaller sized gonad lobes compromising ‘A’ grade yield efficiency.

### **3.5.3 Harvest strategies to improve roe quality**

The results presented here have shown seasonality, age/size and habitat to be the most important factors in driving roe quality of *Centrostephanus rodgersii* in Tasmania. Overall roe quality may be improved by harvesting sea urchins <100mm (~age between 8-18 yrs) and selecting areas that are proportionally dominated by kelp. Interestingly, size distribution of sea urchins measured from commercial catch peaked between 90-100mm. However, a large proportion of catch was greater than 100mm, and therefore commercial fishers could greatly benefit from narrowing the overall total size frequency to not include sea urchins >100mm. Overall, a high proportion of individuals under 100mm scored well enough to be categorised as ‘A’ grade. On the other hand, there was a large degree of variation in roe quality for sea urchins >100mm where most performed poorly. Therefore, it is possible that if harvest operations targeted individuals under 100mm this strategy would increase the proportion of high quality roe in the catch. Furthermore, commercial fishing operations may benefit by targeting fishing grounds

in the southern latitudes of the east coast of Tasmania where younger individuals are distributed (as a function of range extension *see* Ling and Johnson 2009, Ling et al. 2009b).

Other strategies include repeated spatially discrete harvesting that may reduce mean age distribution and increase the proportion of younger individuals. This effect known as ‘fish down’ is typically considered deleterious to a natural population through a reduction in highly fecund biomass. However, in this case removing older and potentially dominant individuals could increase the proportion of younger individuals with high quality roe. Another benefit of removing large individuals is the potential of reducing the intra-specific dynamics that may be playing a role and limiting the movement and foraging potential of smaller individuals. Size specific movement patterns investigated by Dumont et al. (2007) for *Strongylocentrotus droebachiensis* showed that large sea urchin movement patterns are greater than that of small sea urchins. It is possible that similar intraspecific dynamics may be playing a role with *Centrostephanus rodgersii* in Tasmania and this phenomenon could potentially be exploited to improve overall roe quality. Targeted spatial harvesting would not only theoretically reduce the demographic of a population but also reduce the potential for acute overgrazing caused by high *C. rodgersii* densities (Tracey et al. 2014) thereby increasing food availability. This effect may increase the proportion of macroalgal gut content which is shown here to be a significant driver of high roe quality. Habitat type was highly representative of proportion macroalgal gut content, where sea urchins collected from kelp and incipient barrens were found to have the highest proportion (%) of macroalgae when compared to barrens habitat. This indicates that diminished roe quality in barrens habitat is most likely due to the poor nutritional value and food availability.

Overall, significant improvements in roe quality are possible by tailoring harvest operations around certain environmental and biological variables. If tailored harvest operations were to improve economic viability of this fledging fishery, there is significant scope for extensive development along the east coast of Tasmania.

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## **Chapter 4: General discussion**

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#### **4.1 Reproduction of *Centrostephanus rodgersii* in Tasmania**

Samples of *Centrostephanus rodgersii* collected during 2014-15 followed a similar pattern of gonad development described in Ling et al. (2008). *C. rodgersii* gonads were found to condition through late summer and spring and grow to their largest size during June and July prior to a sharp decline throughout August and September. This gonad development pattern observed in Tasmania followed a similar trend to that described by King et al. (1994) and Byrne et al. (1998) in the native home-range of NSW.

Histological examination showed detailed changes in reproductive biology throughout the study period and gametogenic patterns were found to be slightly different across spatial gradients within Tasmania. In general, gametogenic changes were slightly delayed with increasing poleward distribution. Spawning was observed to occur through August and September although there was evidence of individual periodic spawning until November. After spawning, nutritive phagocytes (NP's) were observed to slowly develop and by March/April were dominating the lumen. Rapid gametogenesis transformed NP's to reproductive cells over May, June and July in preparation for spawning.

When compared to the native home range of NSW, the overall gametogenic patterns of *Centrostephanus rodgersii* in Tasmania were slightly delayed with spawning occurring ~1-2 months later. This confirmed the hypothesis that *C. rodgersii* patterns observed in NSW are different in the Tasmanian range extension area. In general, gametogenesis in sea urchins is typically triggered by water temperatures reflective of season coupled with photoperiod and particular phases of the lunar cycle. However, across spatial scales (i.e. NSW to Tasmania) these gametogenic triggers can be fundamentally different and therefore information on gametogenic cycles for *C. rodgersii* from NSW may not be applicable to Tasmania. Overall, determining the reproductive stages and therefore temporal reproductive output is critical to optimising harvest operations of *Centrostephanus rodgersii* in Tasmania. Specific correlations between roe quality and reproductive biology are defined here for harvest operations to be optimized.

## 4.2 Factors affecting roe quality

As expected, seasonality had a significant influence on roe quality. However, when seasonality is usually considered in harvest operations it is about maximizing the volume of product harvested per unit effort. Here we found that large roe (at the end of the gametogenic cycle) was of poor quality when compared to early in the season. Overall, it was found that high proportions of NP's produce the best quality sea urchin roe in *C. rodgersii* (i.e. stage II, growing). Therefore, fishing effort may be targeted around this part of the reproductive cycle. It should be noted that during this period the recovery of roe product (% gonad/total body weight) is not as high as fully developed stage IV premature and V mature gonads. This means that the return of roe weight to commercial fisherman and processors will be less when compared to the later stages. However, later reproductive stages are of much less quality as they represent time periods with less nutritive phagocytes and therefore the price per kilo may be much less compared with early reproductive stages. If fishers and processors are targeting high end markets where prices can attract ~AUD \$300-\$400/kg, then potentially receiving less product that is ~10-fold more valuable is more economically viable.

During the early stages of gametogenesis when roe quality is highest, NP's develop and contain all the necessary biochemical components to partition to developed gametes. At this time, glycogen is dominant and can account for 20-40% carbohydrate per g DW (Zalutskaya et al. 1986; Zalutskaya 1988). However, once gametogenic differentiation is initiated, glycogen content of the gonads declines. Glycogen is a taste active compound related to 'sweetness' and targeted harvesting during early periods of the reproductive cycle when nutritive phagocytes dominate the lumen may increase the probability of harvesting sea urchins that taste sweet. *Centrostephanus rodgersii* has a reputation for being notoriously bitter at certain times and further understanding of these processes is critical to consistently supply of quality product.

Interestingly, gender was found to significantly affect the chances of harvesting 'A' grade roe. Phillips et al. (2009) observed a similar trend where males outperformed females across most roe quality metrics. As ova develop they increase in size and grow larger than spermatozoa when fully mature (Pecorino et

al. 2013). It is postulated that the larger size of ova is contributing to poor granularity and texture within roe of *Centrostephanus rodgersii*. Unfortunately, sea urchins are sexually monospecific and therefore external gender identification is not possible and cannot be considered in harvest strategies.

In the current study, habitat was found to significantly affect the odds of harvesting high quality roe. The two habitat factors used in this study were highly reflective of gut content where the kelp habitat displayed higher proportions of macroalgae in the gut. Echinoids utilise one of the most basic anatomical structures in the animal kingdom and their gonad is the only nutritive store. When nutritive supply becomes limiting, sea urchins will draw on this energy reserve for basic biological function thus severely impacting roe quality. Variation in food supply over the reproductive cycle influences the allocation of resources to somatic and gonadal growth (Munk 1992) and numerous field and laboratory studies have demonstrated that reproduction in sea urchins is directly related to the quantity and quality of available food (e.g. Larson et al. 1980; Thompson 1983; Russell 1998; Guillou and Lumingas 1999; Guillou et al. 2000; Vadas 2000).

In the natural environment, different levels of nutrition can be attributed to contrasting sea urchin habitat types (barrens/kelp). What distinguishes and characterises these two habitat types is the level and complexity of algal cover, which is ultimately reflective of food availability and quality for sea urchins. In 'barrens' habitat, food is limited in availability and quality and therefore roe quality is likely to be low. Conversely, sea urchins in 'kelp' habitats are likely to have access to large quantities of diverse macroalgae and therefore roe quality is expected to be higher when compared to 'barrens'.

In Tasmania and NSW, commercial sea urchin fishers avoid catching individuals in 'barrens' where the roe quality is generally poor and unfit for market. Typically, fishing effort is focused on 'fringe' habitat where 'kelp' intersects 'barrens'. Here, commercial fishers can harvest with confidence that sea urchins have had access to quality food and therefore roe quality is more probable to be high. Ideally, a regulated commercial fishery is an extractive process that over time reduces population densities to obtain a maximum sustainable yield. Consistent commercial fishing in a specific area can reduce densities and increase food availability for residual sea urchins. The recovery of macroalgal beds after sea urchin

removal is well documented (Agatsuma et al. 1998; Hill et al. 2003; Ling 2008; Johnson et al. 2014) and therefore it is logical to assume that food availability and quality will be increased for remaining sea urchins post commercial fishing. Blount et al. (2002) examined the enhancement of roe quality in *Centrostephanus rodgersii* by reducing density. The study found significant improvements in roe quality (colour and yield) in response to reductions of *C. rodgersii* residing in barrens over relatively short periods of time. In addition, the same study investigated transplanting individuals (*C. rodgersii*) from 'barrens' into kelp habitats to enhance roe quality and found roe yield significantly increased after 3 and 12 months post treatment.

A large proportion of the *Centrostephanus rodgersii* population exists in barrens habitat and therefore, a large proportion of the resource is potentially of poor quality. Defining the distance from kelp where commercial fishers can harvest and confidently collect individuals with a high proportion of quality roe will further the biomass available to commercial fisher, particularly in NSW where barrens occur in shallower areas (Andrew et al. 1998). Furthermore, defining how drift algae plays a role in nutrition of *C. rodgersii* in 'barrens' habitat can potentially provide understanding for harvesting high quality roe from this habitat.

Age was also found to significantly affect the odds ratio of harvesting high quality roe whereby younger individuals showed more favorable market characteristics. However, there is limited information in the literature regarding the correlation of sea urchin roe quality and age. Although some limited studies suggest that age is negatively correlated with roe quality (e.g. Sanderson et al. 1996, Agatsuma et al. 2003) whereby larger older individuals produced poorer quality roe.

In heavily exploited fishery populations, large old individuals are generally eliminated more rapidly because they are exposed to size-selective fishing mortality. In this situation, population fecundity not only declines as a consequence of reduced abundance of spawners, but also due to the disproportionate reduction in large, highly fecund individuals. In this context, any reduction of large and highly fecund sea urchins through commercial fishing typically has a deleterious effect for a stock or population (Tegner & Dayton, 1977). However, in the case of the *Centrostephanus rodgersii* fishery in Tasmania,

the reduction of highly fecund large (older) individuals would presumably have no effect on recruitment as the stock is solely reliant on spawning aggregations in NSW (Johnson et al. 2004). Furthermore, the removal of older individuals may increase the probability of harvesting younger sea urchins that are potentially producing higher quality roe. This scenario is unique to this fledgling fishery, and as such may play a role in any future management approaches.

Developing fisheries tend to experience what is known as a ‘fish-down’ period during the early years of operation. The ‘fish-down’ phenomenon is a term coined to represent the effect of high initial production due to harvesting biomass of virgin stocks (Andrew et al. 2002). This pattern is typified by large catches during this development phase whereby older and larger animals are harvested with the remaining population shifting to a younger age structure. For a newly emerging sea urchin fishery, this means that harvesting a large proportion of older animals, where roe quality may be relatively poor, will have the effect of shifting the populations structure toward a younger overall demographic (dependent on recruitment), which may ultimately yield improved roe quality.

During the early years of the New South Wales and Tasmanian *Centrostephanus rodgersii* fisheries, large-scale harvesting did not occur and the majority of initial catches were typified by poor quality roe/product. In this sense, it is likely that catches consisted of older animals containing poor quality roe and therefore a ‘fish-down’ did not occur. However, there is strong anecdotal evidence from New South Wales and Tasmanian *C. rodgersii* fisheries to suggest that improved gonad quality is achievable after sustained fishing and removal of older animals at certain sites.

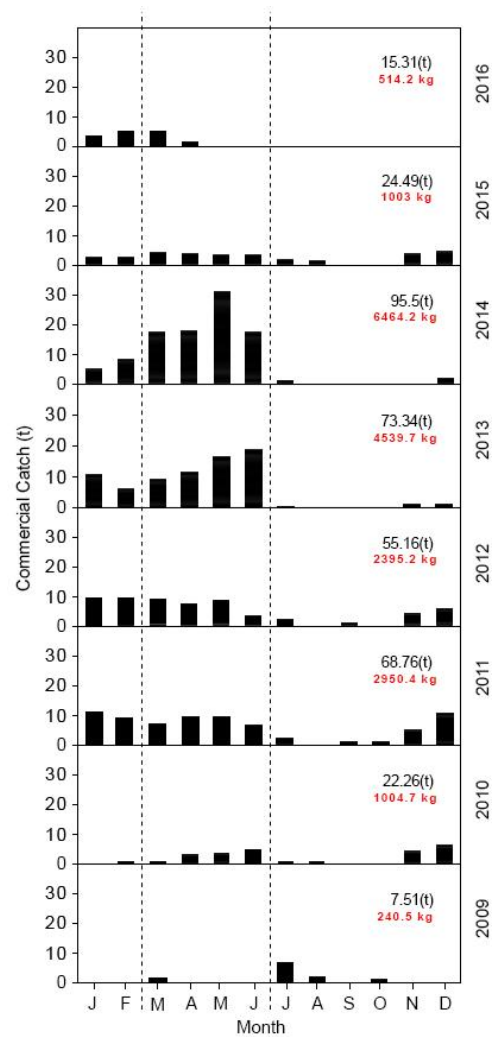
Interestingly, the *Centrostephanus rodgersii* fishery in Tasmania is a direct result of climate-driven range extension where potentially different age population structures exist relative to the historical home range of NSW. There is potential that the newly extended range may be more efficient, in terms of harvesting younger individuals. In addition, areas in Tasmania that have not been harvested would also need to be investigated to determine if there is scope to expand the current fishery in a viable manner (i.e. the level of fishing required before high quality roe were being harvested consistently), or target areas that have a younger population structure such as the more southern latitudes (i.e. Fortescue Bay).



### 4.3 Expanding the current fishery

Currently the Tasmanian *Centrostephanus rodgersii* fishery is harvesting only small amounts (15.31t in 2016) of sea urchins. New information presented here (i.e. temporal roe quality patterns and defined predictors), suggests fishing effort should be focused between May and June to optimise recovery and quality. From a business perspective this obviously creates challenges as capital is unutilised for most of the year. It may be possible or necessary to overcome this barrier by integrating sea urchin processing with some other form of processing that occurs at a different time of the year (potentially *Heliocidaris erythrograma*). Interestingly the fishery appeared to evolve towards the optimal HW from 2008 to 2014 but this pattern disappeared in the 2015-16 commercial harvest season; with catch spread through the year. This may indicate the loss of knowledge from the fishery, when the major processing facility closed due to business management reasons. The key point is that based on these findings, the fishery can become significantly improved by optimising its HW to within the months of May and June.

There has been a seasonal shift in commercial landings of *Centrostephanus rodgersii* in Tasmania since the inception of the fishery in 2009. Initially, catch and effort was spread throughout the calendar year and landings were small (7.5t in 2009). Annual catch increased in successive years until a peak in 2014 of 95.5t when landings occurred mainly in the first six months of the year in an attempt to improve roe quality and recovery (Figure 20). After 2014, total landings dropped to 24.9t when the largest *C. rodgersii* processing company closed.



**Figure 20:** Monthly *Centrostephanus rodgersii* commercial catch landed throughout the calendar year between 2009 and April 2016. In between dotted lines denotes observed periods of high proportion ‘A’ grade roe quality. Total annual tonnage is indicated in black and kilograms of forecasted ‘A’ grade roe is denoted in red and calculated as proportion on ‘A’ grade roe multiplied by total monthly kilograms harvested multiplied by GI (ratio of roe to body weight). Monthly totals were summed to estimate annual total ‘A’ grade roe harvested.

Roe recovery from *Centrostephanus rodgersii* is low (max 19% body weight in collected samples) compared to other commercially harvested sea urchins (Saito 1989), especially for ‘A grade’ roe as presented in this study. At the peak of this fledgling fishery during 2014, 95.5t were harvested yielding an estimated 6.5t of ‘A’ grade roe or a recovery rate of almost 7%. This improvement was due to the majority of the catch being landed between the months of May and June (peak quality months, Figure

20). This represents a high proportion of catch resulting in ‘A’ grade roe yet the major processing company could not maintain a viable business.

Australia has large stocks of sea urchins (*Centrostephanus* spp. & *Heliocidaris* spp.); however, delivering gonads of suitable quality to distant, high-value Asian markets has proven difficult because of a lack of understanding of the factors controlling shelf-life and quality of sea urchin gonads. The value of the gonads is closely linked to their appearance and a defect known as ‘melting’, which is a loss of surface granular definition and a reduction in water holding capacity. This phenomenon is a major indicator of the end of shelf life (Verachia et al. 2012). Gonads recovered from *C. rodgersii* have traditionally been mainly sold on the domestic market as the product has a poor reputation in the international market place because of inconsistency in appearance, colour, taste (bitterness) and yield.

In Japan, it is a common practise to hold sea urchins at 0°C for 12 h prior to processing, as this is believed to increase the subsequent shelf-life of the recovered gonads, because of a slowing down of the sea urchin’s metabolism prior to processing. Scientific data on factors affecting the shelf-life of sea urchin gonads, however, are limited. In Australia, sea urchins are often harvested from remote locations, and it is a common practise to hold live in air on the deck of the fishing boat for several days prior to processing. In the present study, we show that time of water was significantly affecting roe quality. In general, post-harvest handling and processing have been shown to influence roe quality and shelf-life of the New Zealand sea urchin (*Evechinus chloroticus*) (Verachia et al. 2012, 2013). Surprisingly, published information is limited for other sea urchin species. In Australia, sea urchins can be harvested significant distances from the processing facilities and as such, may be held in ambient air temperatures for 24hrs to 48hrs. While the impact of this standard commercial practise has not been reported for *Centrostephanus rodgersii*, it is likely to be deleterious for roe quality.

#### **4.4 Potential for *Centrostephanus rodgersii* into global markets**

Traditionally, the seasonal availability of sea urchin products in major Asian markets (namely Japan) has been closely linked to the reproductive cycle of local species (*Strongylocentrotus intermedius* and

*S. nudus*). These species comprise 80% of total supply to the domestic Japanese market and are mostly harvested between March and August. However, more recently the Japanese population has come to expect year round supply of sea urchin products and the highest prices are usually paid in January and September, reflecting the low availability of domestic roe during these months (Sonu, 1995). This coupled with declining local stocks has driven the Japanese market to seek alternative sources sea urchin products. To fill the gap in demand, sea urchin fisheries were developed Canada, USA, Mexico, Russia South Korea, and Chile.

Annual output of the world's sea urchin fisheries has steadily increased since its inception in the early 1950's with an increase in demand and harvesting technologies. The total world catch peaked in 1995 at ~120,000t (Andrew et al. 2002; Keesing and Hall 1998). Since then, catches have declined substantially and plateaued to current levels of 60,000-70,000t per annum (FAO fishstat 2012). Overall, the future market direction for imported sea urchins to Japan depends largely on the Japanese sea urchin harvest. Because the domestic harvest is not likely to increase in the short term, increased export of sea urchin products from around the globe has significant potential.

#### **4.5 Commercial fishing to control *Centrostephanus rodgersii* overgrazing**

The east coast of Australia has recently undergone change and displacement of typical marine isotherm distribution as a result of a strengthening East Australian Current (EAC) (Ridgway and Godfrey 1996, Thresher et al. 2004, Cai et al. 2005, Ridgway 2007). This regime shift in ocean temperature has resulted in southward latitudinal range extension for some marine species through changes in dispersal patterns and previously unsuitable areas becoming habitable (e.g. Poloczanska et al. 2007, Pitt et al. 2010, Last et al. 2011, Wernberg et al. 2011). If climate projection models are accurate, it is expected that further southward penetration of warm EAC water will supersede the current distribution thereby advancing the southern extremes of distribution for opportunistic range expanding species. Species range-contractions and range-extensions as a result of climate change are being increasingly documented with clear effects for coastal fisheries (Salinger 2012; Melnychuk 2014). Impacts include either declines in

local fisheries for cold-water species, or potential opportunities for the creation of new fisheries through warmer-water species extending their distribution along latitudinal gradients.

It is now apparent that there have been significant and ongoing recruitment events of *Centrostephanus rodgersii* into Tasmania and recent distributions, population dynamics, and age structure of the sea urchin correlates strongly with patterns in recent climate change for eastern Tasmania (Ling et al. 2008; Ling and Johnson 2009, Johnson et al. 2011; 2012). Currently there is no evidence that Tasmanian *C. rodgersii* meta- populations are undergoing self-recruitment success. However, it has been found that viable gametes are produced by these populations, but development of larvae is poor at water temperatures below 12°C (Ling et al. 2008). This supports the theory that future recruitment may occur in Tasmania from not only mainland populations but also Tasmanian populations if the current increasing trend of the EAC continues.

*Centrostephanus rodgersii* herbivory on shallow temperate rocky reef systems across eastern Australia is unsurpassed and is frequently implicated with major shifts in benthic ecological structure (Fletcher 1987; Andrew and Underwood 1989, 1993; Ling et al. 2008, 2009). Intensive non-discriminant overgrazing of productive kelp beds by *C. rodgersii* triggers a collapse of biogenic habitat leading to sea urchin ‘barrens’ devoid of foliose macro algal habitat. *C. rodgersii* barrens are typically characterised by lessened habitat complexity, productivity and biodiversity when compared to adjacent macroalgal beds that support complex ecological systems. This forward phase shift to barrens habitat is often met with deleterious consequences for the complex ecosystems that are supported by kelp bed habitats. Reefs whereby the entire kelp bed ecosystem has collapsed are defined as extensive or widespread barrens and are formed by coalescence of smaller patch barrens which are termed ‘incipient barrens’ (*sensu* Johnson et al. 2005, 2011; Flukes et al. 2012). In Tasmania, extensive barrens are known to occur at several sites around the Kent group of Islands (Bass Strait) and the St Helens area (eastern mainland Tasmania); however they become less prominent in the southern extremes of distribution where only incipient barrens currently occur (Johnson et al. 2011). The potential for the creation of an

alternative stable state along the entire east coast of Tasmania as observed in NSW is significant and directly threatens the ecological integrity of these habitats.

Globally, the formation of sea urchin-dominated extensive barrens and the threat of further habitat destruction as a whole is not a new concept (Mann 1977, Ling et al. 2014). However, the phenomenon is relatively new in Tasmania. This transition of regime shift to complete barrens can not only effect ecological function and diversity, but also directly implicate wild commercial fisheries that are reliant on kelp beds for recruitment and habitat including. *Inter alia*, there has been much research on *Centrostephanus rodgersii* in Tasmania and more broadly Australia to assess potential management strategies that may be used to minimise destructive overgrazing. Several workshops have been convened linking stakeholders (Government, researchers, fisheries industries, conservation groups, and recreational fisheries) in a collaborative forum to discuss the economics, likelihood of success and implementation of potential strategies. The first workshop was held in 2006 and the second in 2014. Typically, the workshops have focused on demonstrating potential strategies, not implementing or determining specifications. To date the most promising strategies have included increasing the biomass of large predatory species (namely southern rock lobster *Jasus edwardsii*) (Johnson et al. 2014), systematic direct culling (Tracey et al. 2014), and the further development of the commercial fishing industry for the species.

Tracey et al. (2014) investigated an industry implemented, spatially discrete eradication/control program for *Centrostephanus rodgersii* in Tasmania. The study tested the efficacy of systematic culling in targeted areas and found that systematic culling in incipient barrens habitat effectively reduced the mean density of sea urchins from 1.51 m<sup>-2</sup> prior to intervention to 0.13m<sup>-2</sup> sea urchins when resurveyed 12 months post culling exercise. Due to heterogeneous distribution of sea urchins within the habitat and restricted individual home ranges (Flukes et al. 2012), the effect of systemic culling appears that recovery of kelp bed habitats is possible in the short term, however, will be dependent on recruitment and subsequent culling. However, culling sea urchins on a large scale is potentially not viable given the extensive dive hours required. Although, if there were a cost recovery mechanism such as for roe

products, then potentially this option may be viable. Therefore, commercial fishing has the potential to be the most direct and economically viable method of control for minimising destructive overgrazing. Direct removal is quantified and potentially large scale provided the fishery expands.

While it is likely that there is no 'silver bullet' to this issue, the best chance for success is a suite of control measures that overlap and complement each other. Ultimately, the economics and likely success of each strategy must be considered.

## Conclusion

Predicting roe quality of *Centrostephanus rodgersii* is inherently problematic because sea urchins do not display any external quality features. However, there are proxy characteristics that can be targeted for improvements in the likelihood of harvesting high quality roe. Here we have shown that targeting periods when the reproductive biology is favorable to roe quality and potentially incorporating specific size/age and habitat characteristics along with post-harvest handling procedures can significantly improve the proportion of high quality roe harvested. This research presents detailed information that commercial harvesters and processors can consider when determining harvest operations for improvements in the fishery and overall economic viability.

The *Centrostephanus rodgersii* fishery in Tasmania is a relatively new resource and as such there is limited overall information. Further research on cyclical biochemical composition of roe may be advantageous for the fishery to determine favorable profiles to target *C. rodgersii* with the most positive taste characteristics. Also, current information on age population structure along the distribution in Tasmania could also provide information to determine harvest planning and target areas where high densities of young individuals occur. Furthermore, information on potential strategies to harvest ‘barrens’ areas could increase available *C. rodgersii* to commercial harvesters. This would involve information on nutritional requirements for high quality roe and potential available sources (i.e. distance from kelp habitat and the potential for ‘drift’ macroalgae that has been dislodged during significant swell events). In addition, it has been shown that transplanting individuals from ‘barrens’ habitat can improve the quality of roe, however, the economics around this activity are yet to be determined. Harvesting *C. rodgersii* from ‘barrens’ habitat has the added advantage of potential remediation of these habitats. However, current and potential future commercial harvesting is mostly focused in ‘kelp’ habitats or areas of small barrens patches termed ‘incipient barrens’ until further information suggests otherwise.

Overall, this research has determined that there are certain biological and environmental parameters that should be considered when planning and undertaking harvest operations for high quality roe. The arrival



of *Centrostephanus rodgersii* in Tasmania has presented economic opportunity and ecological challenges, however, further understanding of the drivers of roe quality may increase development and commercial harvesting participants in the fishery to maximise potential of this resource.

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